EXPERIMENTS CONCERNING THE MOLECULAR EVOLUTION OF THE ALLOTETRAPLOID *PENNISETUM PURPUREUM* (NAPIERGRASS)

By

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This dissertation is dedicated to my mother Verna, my brother Jimmy, my sisters Lynda, Beth, and Linda, my stepfather Jim, my grandmother Thelma, my grandfather Howard and my great aunt and uncle Verna and Vincent. Besides my blood family, this dissertation is dedicated to my spiritual families who are teaching me to live life on life's terms and to strive for my potential. I also dedicate this dissertation to Dave who has consistantly been there for me and has been guiding me through the steps.

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS iii

ABSTRACT v

CHAPTER 1 INTRODUCTION 1

CHAPTER 2 MATERIALS AND METHODS 19

DNA Isolation, RNA Isolation, Digestion, and Fractionation 19

Southern Blotting, RNA Blotting, Probe Labeling, and Hybridizations 22

Genomic and cDNA Cloning 25

Sucrose Synthase Isolation and Antibody Production . 25

Copy Number Determination 25

DNA Sequencing 27

Isozyme Electrophoresis and Staining 28

CHAPTER 3 RESULTS OF PROGENITOR SYUDY 30

CHAPTER 4 RESULTS OF SUCROSE SYNTHASE COMPARISONS . . . 95

CHAPTER 5 DISCUSSION 150

REFERENCE LIST 167

BIOGRAPHICAL SKETCH 179

Abstract of Thesis Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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This study was undertaken to obtain molecular evidence concerning the identification of the progenitors of the allotetraploid *Pennisetum purpureum* Schumach (napiergrass) with particular emphasis on sucrose synthase coding sequences of *P. purpureum* and *P. glaucum* (L.) R. Br. (pearl millet). Published cytological and crossability evidence suggests that *P. glaucum* (AA) is one of the progenitors of *P. purpureum* (A'A'BB). The data presented here suggest that *P. squamulatum* may share in common both the A' and B genomes of *P. purpureum*. Average or consensus sequences were determined for the 140 bp and 160 bp *Kpn* I families of tandemly arrayed repetitive sequences from *P. purpureum* and *P. squamulatum*. Average sequences were determined for the *P. glaucum* 140 bp *Kpn* I family and the diploid *P. hohenackeri* Hochst. ex Steud. 160 bp *Kpn* I family. *P. purpureum* and *P. squamulatum* share similar

restriction fragment length polymorphisms (RFLP). Isozyme phenotypes discussed here show a close relationship between P. purpureum, P. squamulatum, and P. glaucum. The combination of the evidence presented here with previously published evidence including crossability, isozyme phenotypes, and cytoplasmic genome similarities suggests that P. purpureum evolved from the interspecific hybridization of P. glaucum with P. squamulatum. The other possibility is that P. purpureum and P. squamulatum share common progenitors and that the interspecific hybridizations which gave rise to these plants occurred at approximately the same time period. P. purpureum diverged from P. squamulatum at a later date than their divergence from P. glaucum. The major sucrose synthase proteins in P. purpureum and P. glaucum root appear slightly smaller than the 88,000 mol wt maize sucrose synthase proteins. A sucrose synthase genomic sequence from P. purpureum was shown to have similar gene structure as compared to the Sus gene from maize. The cDNA encoded by this P. purpureum sucrose synthase gene as well as a P. glaucum sucrose synthase cDNA was sequenced. These two cDNAs exhibit 98.6% homology from exon 5 through the polyadenylation site in the P. purpureum cDNA. Another genomic sequence from P. purpureum having similarity to the 5′ end of Sh1 has been cloned. High copy number poly A^+ RNA of less than 2.0 kb having similarity only to the 5′ end of sucrose synthase coding sequences was also identified.

CHAPTER 1
INTRODUCTION

Tandemly arrayed repetitive sequences appear to evolve in concerted evolution (Dover 1982). These repeated DNA sequences remain homogenous within a species over evolutionary time. The rate of fixation of the sequence is greater than the rate of divergence (Dover 1982; Arnheim 1983). Molecular drive results in the fixation of a particular repeated sequence. This fixation of variants in a population is not predicted by the genetics of natural selection and genetic drift (Dover 1982). Two major mechanisms have been proposed to participate in concerted evolution; unequal crossing over (Smith 1976) and gene conversion (Ohta 1977; Nagylaki and Petes 1982).

Mechanisms allowing the proliferation of tandemly arrayed repetitive sequences have been postulated. Tandemly arrayed repetitive sequences are generally located in the constitutive heterochromatin of chromosomes (John and Miklos 1979; Appels et al. 1981; Bedbrook et al. 1980). These heterochromatic regions of chromosomes have low recombinational rates and hence reduced rates of unequal crossing-over (Miklos 1985). Stephan (1989), using computer programming, suggests that the crossing-over rate in heterochromatin is too low to cause the proliferation of tandemly arrayed repetitive sequences.

Slipped-strand mispairing, or replication slippage, may be a major mechanism behind concerted evolution of repeated sequences (Levinson and Gutman 1987). Based upon computer modeling, Stephan (1989) has proposed that the creation of nucleotide periodicities involves not only unequal crossing-over, but an amplification process as well. This amplification process may consist of replication slippage and rolling circle replication. The evidence for rolling circle replication is the appearance of extrachromosomal circular satellite DNAs (reviewed by Walsh 1985) suggesting an extrachromosomal amplification process such as rolling circle replication (Hourcade et al. 1973).

The function of repetitive DNA is an unresolved topic. Repetitive DNA has been termed selfish DNA. Doolittle and Sapienza (1980) referred to prokaryotic transposable elements and eukaryotic middle repetitive sequences as selfish DNA; and suggested that selfish DNA promotes its own spread through a population at the expense of the evolutionary fitness of the population. Orgel and Crick (1980) stated that selfish DNA arises as a sequence spreads through a genome while making no significant contribution to the phenotype. These authors referred to selfish DNA as repetitive DNA, introns, and segments of DNA between genes. Dover (1980) questioned the concept that selfish DNA is not involved in phenotypic selection; however, he did agree that selfish DNA may not have an intrinsic function. He suggested that selfish DNA may give

some specific and common form to closely related genomes. Many workers have suggested varied functions of repetitive sequences. Bostock (1980) proposed that the association of constitutive heterochromatin with satellite DNA, or tandemly arrayed repetitive sequences, suggests possible functions of these repetitive elements. He classified potential functions into four broad categories: chromosome structure, cell metabolism, homologous pairing of chromosomes, and promotion of chromosome rearrangements or recombination. Miklos (1985) has reviewed proposed functions of tandemly arrayed repetitive sequences. The functions which have been proposed include determination of centromere strength, chromosome pairing, recombination, three dimensional architecture of the nucleus, genomic reorganization, and speciation. Also mentioned is a second class of proponents suggesting that these repeated sequences are byproducts of molecular mechanisms involved in DNA replication and recombination. In spite of advances in molecular biology, the role of repetitive sequences continues to elude investigators.

In plants, tandemly arrayed repetitive sequences are often termed highly repetitive sequences. Highly repetitive sequences have been classified as those sequences with a copy number greater than 100,000 per genome (McIntyre et al. 1988). There are, however, examples of moderately repetitive sequences which are tandemly arranged (Zhao et al. 1989; Hallden et al. 1987; Shmookler-Reis et al. 1981). These

tandemly arrayed repetitive sequences are often termed satellite DNA (Ganal et al. 1986; Peacock et al. 1981) because they may be separated from the bulk of nuclear DNA by equilibrium sedimentation in density gradients (Kit 1961). The term satellite DNA is a misnomer since not all tandemly arrayed repetitive sequences are AT rich and thus cannot be separated in a density gradient (Koukalova et al. 1989). Repeat sizes of tandemly arrayed repetitive sequences in plants are usually in the 160 to 200 base pair range (Schweizer et al. 1988; Junghans and Metzlaff 1988; Dennis and Peacock 1984). Repeat sizes in the 350 base pair range have also been reported (Ganal and Hemleben 1986; Ganal and Hemleben 1988). Tandem repetitive elements in constitutive heterochromatin are commonly methylated (Deuming 1981; Shmookler-Reis 1981; Ganal and Hemleben 1988). The percentage of plant genomes consisting of characterized repetitive element families varies: the highly repeated satellite DNA of Cucumis sativus composes 20-30% of the nuclear DNA (Ganal et al. 1986), the GC rich satellite DNA in Scilla siberica accounts for 20% of the nuclear genome (Deumling 1981), the Hind III family of Brassica campestris accounts for 15% of the genome (Lakshmikumaran and Ranade 1990), the 375 bp tandemly repeated sequences of Allium cepa constitutes 4% of the genome (Barnes et al. 1985), and the Bam HI family in Nicotiana tabacum accounts for 2% of the nuclear genome (Koukalova et al. 1989). Variation in DNA content between closely related

species is at least partially due to differences in the amount of tandemly arrayed repetitive sequences (Bennett et al. 1977; Geever et al. 1989).

In plants there are few examples of characterized tandemly arrayed repetitive sequences. Most of the examples include Scilla siberica (Deumling 1981), Vicia faba (Kato et al. 1984), Allium cepa (Barnes et al. 1985), Triticum species (Rayburn and Gill 1988), Oryza species (Zhao et al. 1989), Secale cereale and Secale silvestre (Bedbrook et al. 1980). Secale cereale and Agropyron cristatum (Xin and Appels 1988), Lycopersicon esculentum and Solanum acaule (Schweizer et al. 1988), Hordeum vulgare (Junghans and Metzlaff 1988), Zea mays and its relatives (Dennis and Peacock 1984), Cucurbita species (Leclerc and Siegel 1987; Ganal and Hemleben 1986), Cucumis sativus L. (Ganal and Hemleben 1988), Nicotiana tabacum (Koukalova et al. 1989), and several species within the family Brassicaceae (Hallden et al. 1987).

Tandemly arrayed repetitive sequences in plants have been utilized for various purposes; most of these involve investigating the relatedness of species. For example, in the family Brassicaceae, a 175 bp sequence from Brassica napus hybridized to DNA from six genera within that family. One of the species hybridizing to this repeat was in a different tribe than Brassica napus (Hallden et al. 1987). In Cucumis sativus L. there are three types of tandemly arrayed repetitive sequences of around 180 bp (Ganal et al. 1986).

Also present in this species is a 360 bp repetitive element that contains a 180 bp segment related to the above three types of sequences. There is a sequence in the related species Cucumis melo similar to the cucumber 360 bp repeat (Ganal and Hemleben 1988). Major tandemly arrayed repetitive sequence families from two Cucurbita species, C. pepo and C. maxima were cloned and sequenced. These repetitive elements exhibited no cross homology. However, the C. pepo repeat was present in low amounts in the C. maxima genome and vice versa indicating a common ancestor contained both of these sequences prior to speciation (Ganal and Hemleben 1986). The major tandemly repeated sequences in maize heterochromatin were shown by hybridization and sequencing to be more closely related to teosinte than to Tripsacum dactyloides thus suggesting maize was derived from teosinte (Dennis and Peacock 1984). Tandem repeats from Lycopersicon esculentum and Solanum acaule were used to identify hybrids from protoplast fusions between these two species. The tomato repeat is also present in other Lycopersicon species (Schweizer et al. 1988). A common 350 bp repeat found in Secale cereale and Agropyron cristatum terminal heterochromatin was reported to be an example of parallel amplification and not an indication of relatedness between these two species. The S. cereale and A. cristatum species differed at their rDNA intergenic spacer regions, 5S DNA loci, and also differed in morphological characters, chromosome pairing, and isozyme patterns (Xin and Appels

1988). In the Oryza species genome specific tandemly arrayed repetitive sequences are specific based upon hybridizational intensities; these probes can be used for classifying unknown species (Zhao et al. 1989). Most other published work on plant tandemly arrayed repetitive sequences involves only the characterization of those families.

Another repetitive sequence family used in this evolutionary study is ribosomal DNA, or rDNA. Structural variation in rDNA between species can provide useful information concerning the points of divergence of related species (Springer et al. 1989; Burr et al. 1983; Cordesse et al. 1990). Ribosomal DNA repeat structure has been well characterized (Long and Dawid 1980; Appels and Honeycutt 1986). The 17S, 5.8S, and 26S rDNA genes are transcribed as one unit and then cleaved to yield mature rRNA (Perry 1976). Between each set of rDNA genes is the intergenic spacer, or IGS (Miller and Beatty 1969). Ribosomal DNA varies in repeat size within and between species (Rogers and Bendich 1987a; Choumane and Heizmann 1988; Molnar et al. 1989; Cordesse et al. 1990). Heterogeneity of rDNA repeat size is present within many species (Jorgensen et al. 1987; Rogers and Bendich 1987; Molnar et al. 1989; Springer et al. 1989; Cordesse et al. 1990) but absent from others (Varsanyi-Breiner et al. 1979; Kavanagh and Timmis 1988; Springer et al. 1989). These repeat sizes range from 7.8 to 18.5 kb (Varsanyi-Breiner 1979; Yakura et al. 1983). Length variation of the rDNA repeat within a

species and/or within a genus has been accounted for by the number of subrepeats within the IGS (Yakura et al. 1984; McMullen et al. 1986; Choumane and Heizmann 1988; Cordesse et al. 1990). In Hordeum species the number of rDNA repeat sizes is equal to the number of nucleolar organizer regions (NOR) or rDNA loci (Molnar et al. 1989). There is only one NOR in Vicia faba but the rDNA repeat length shows considerable heterogeneity; hence in this case the number of rDNA loci is not equal to the rDNA repeat length heterogeneity (Rogers and Bendich 1987b). Allotetraploids in Oryza species may exhibit less rDNA repeat size polymorphisms than their diploid progenitors. This homogenization process of the rDNA loci on nonhomologous chromosomes may occur relatively rapidly (Cordesse et al. 1990). Triticum aestivum exhibits co-evolution of rDNA repeats on nonhomologous chromosomes as well (Dvorak 1982).

The Kpn I tandemly arrayed repetitive sequence families in several Pennisetum species were characterized, along with RFLP analysis, to identify relationships between nuclear genomes of P. purpureum and related species P. glaucum and P. squamulatum. There are several lines of evidence suggesting a relatedness amongst the above three species. P. glaucum (2n=2x=14) is considered to be a progenitor of P. purpureum (2n=4x=28) (Jauhar 1981). When P. glaucum (AA) is crossed with P. purpureum (A'A'BB), the resulting triploid interspecific hybrid (A'AB) contains seven bivalents and seven univalents

suggesting a close relationship between these two species (Harlen 1975; Jaurar 1981). P. squamulatum (2n=6x=54) has been described as an autoallohexaploid (Patil et al. 1961), an allohexaploid (Rangaswamy 1972), and a segmental allohexaploid (Jaurar 1981). An autoallohexaploid contains two different genomes, one of which is in the tetraploid state. An allohexaploid is composed of three different genomes. A segmental allohexaploid exhibits segmental homology between the constituent genomes. P. glaucum can also be crossed with P. squamulatum at a high frequency (Dujardin and Hanna 1989) resulting in sexual and apomictic hybrids (Dujardin and Hanna 1983). Napiergrass and P. squamulatum as well can be crossed (Hanna, unpublished). Double cross hybrids between P. glaucum, P. purpureum, and P. squamulatum indicate a relatedness between the genomes of these three species (Dujardin and Hanna 1984). It is likely that P. squamulatum or P. purpureum genes fully and partially restored male fertility in cytoplasmic male sterile P. glaucum interspecific hybrids thus indicating common genes are present (Dujardin and Hanna 1990). The monomorphic prolamine seed proteins in P. glaucum are present in P. squamulatum and some accessions of P. purpureum (Lagudah and Hanna 1990). Isozyme banding patterns also suggest that P. squamulatum has partial homology to the genomes of P. glaucum and P. purpureum (Lagudah and Hanna 1989). Significant relationships exist between P. purpureum and P. squamulatum

mitochondrial DNAs (mtDNA); both of these mtDNAs are related to P. glaucum mtDNA (Chowdury and Smith 1988).

This study focuses on the close homology of the Kpn I tandemly arrayed repetitive sequence families between P. purpureum, P. squamulatum, and P. glaucum. Also addressed is the similarity between P. purpureum and P. squamulatum in rDNA RFLPs, the common RFLPs when DNA from these two species are probed with napiergrass genomic and cDNA clones, and similar isozyme phenotypes. The unexpected results of this study suggest that P. purpureum is more closely related to P. squamulatum than it is to P. glaucum. When all the evidence is considered, the genomes of the hexaploid P. squamulatum may include the genomes present in the tetraploid P. purpureum. P. purpureum and P. squamulatum may have had common progenitors and arose from independent interspecific hybridization events at similar times. Or, napiergrass (A'A'BB) may have evolved from a cross of P. squamulatum and P. glaucum with both gametes in the reduced state prior to fertilization. The above cross would yield an interspecific hybrid composed mostly of chromosomes from P. squamulatum; this model fits the data presented in this paper.

Another set of experiments involved with studying the molecular evolution of P. purpureum included the cloning and sequencing of sucrose synthase coding sequences. By comparing the sequences of sucrose synthase genes within and outside of

the genus Pennisetum the divergence of P. purpureum was further elucidated.

Sucrose synthase (UDP-glucose: D-fructose-2-glucosyl transferase, EC 2.4.1.13) catalyzes the reversible reaction below:

UDP-glucose + fructose <-----> Sucrose + UDP

(Cardini et al. 1955). The isolation of this enzyme first occurred in wheat germ (Cardini et al. 1955). Originally Cardini et al. (1955) suggested that sucrose synthase led to the formation of sucrose in vivo. By 1957 Turner and Turner proposed that the function of sucrose synthase is to supply UDP-glucose as a glucosyl donor in the synthesis of starch. Experimental evidence supporting the proposed role of sucrose synthase in the degradation of sucrose was initially obtained in sweet corn (De Fekete and Cardini 1964) and in rice (Murata et al. 1966). The experimental evidence was the same for both sweet corn and rice; glucose-C^{14} from sucrose was incorporated into starch while the nucleotide diphosphate (NDP), UDP, was shown to be the NDP of choice for the enzyme degrading the sucrose. Since sucrose synthase utilizes UDP in the degradation reaction of sucrose, the authors of both papers suggested that sucrose synthase degrades sucrose instead of synthesizing sucrose. Chourey and Nelson (1976) offered the first genetic evidence supporting the role of sucrose synthase in starch biosynthesis by showing that sh1 (a sucrose synthase deletion mutant was used in this experiment) maize kernels are

deficient in starch. Detailed kinetic and substrate specificity studies of sucrose synthase from various sources (De Fekete and Cardini 1964; Avigad 1964; Avigad et al. 1964; Milner and Avigad 1964, 1965; Avigad 1967; Grimes et al. 1970; Murata 1971; Delmer 1972; Murata 1972; Sharma and Bhatia 1980) suggest that sucrose synthase degrades sucrose mainly in storage tissues. This sucrose degradation occurs at sites lacking in invertase, with intense growth, and with high sucrose concentration (Avigad 1982). The UDP-glucose product from sucrose synthase degradation of sucrose can be used also for cell wall synthesis and storage compounds (Feingold and Avigad 1980). Franck (1979) referred to D-glucose as the major "key building block" used for synthesis of natural products. Pontis (1977) suggested that for heterotrophic organisms whose viability depends on the consumption of organic carbon, most organic constituents have, at one time, been part of a sucrose molecule.

Carbon fixation leading to sucrose biosynthesis differs between C_3 and C_4 photosynthetic types of plants. In C_3 plants the reductive pentose phosphate pathway occurs in the stroma of the chloroplast; CO_2 and inorganic phosphate are converted to triose phosphate and exported to the cytosol for conversion to sucrose (Sicher 1986). With C_4 plants, characteristically tropical grasses, there is an added cycle of carbon fixation The first cycle of carbon fixation occurs in the mesophyll cells. In NADP-malic enzyme (NADP-ME) C_4 plants,

phosphoenolpyruvate is carboxylated to yield oxaloacetate in the cytosol of the mesophyll cells. This oxaloacetate is converted into malate in the chloroplast; the malate is transported to the chloroplast of the bundle sheath cells. The malate is then decarboxylated to yield pyruvate which cycles back to the mesophyll chloroplast to be converted to phosphoenolpyruvate. The CO_2 from malate then enters the reductive pentose phosphate pathway. Sucrose is synthesized in the cytosol of the bundle sheath cells of C_4 type photosynthetic plants (Rees 1987).

The free energy of hydrolysis of sucrose is conserved by the formation of UDP-glucose. The free energy of sucrose hydrolysis is -7000 calories (cal). This free energy is conserved in the formation of UDP-glucose which has a free energy of hydrolysis of the alpha-D-glucopyranosyl phosphate bond of -8000 cal. This energy of the glycosidic linkage of sucrose could be used for the synthesis of other saccharides, starch, callose, or cell wall polysaccharides (Neufeld and Hassid 1963).

Evidence for the direction of the sucrose synthase reaction includes a comparison to sucrose phosphate synthetase, tissue specificity, and acceptor concentration (fructose). Both sucrose synthase and sucrose phosphate synthetase are present in photosynthetic tissue; however, very low activities of sucrose synthase have been detected in leaves (Claussen et al. 1985). In nonphotosynthetic tissue,

sucrose synthase is present while sucrose phosphate synthetase is relatively low in abundance (Avigad 1982). Because of the differential expression of these enzymes a conclusion was reached that sucrose synthase activity is high only in nonphotosynthetic plant tissue (Delmer and Albersheim 1970). Not much attention has been focused on the role of sucrose synthase in leaves because of the above conclusion (Claussen et al. 1985). As discussed, sucrose synthase in nonphotosynthetic tissue such as storage tissue and sink organs likely degrades sucrose yielding UDP-glucose for cellular biosynthesis (Avigad 1982). The direction of the two reactions in photosynthetic tissue could be either yielding sucrose in the forward direction or degrading sucrose in the reverse direction. Synthesis of sucrose by sucrose synthase depends on the availability of free fructose; fructose is barely detectable in intact leaves while present in storage tissues, roots, developing seeds, and exudates (Feingold and Avigad 1980; Avigad 1982). Fructose in the cytoplasm may rapidly be phosphorylated by hexokinase or fructokinases (Turner et al. 1977; Copeland et al. 1978). This phosphorylated form of fructose will serve as a substrate for the sucrose phosphate synthetase reaction (UDP- glucose + Fructose 6-phosphate <-----> sucrose 6-phosphate + UDP + H^+) thus competing with the sucrose synthase reaction leading to sucrose synthesis (Avigad 1982). The sucrose 6-phosphate formed from sucrose phosphate synthetase is hydrolyzed to free

sucrose by sucrose phosphatase; this irreversible pathway could account for the large accumulation of sucrose in many plants (Neufeld and Hassid 1963). In photosynthetic tissue sucrose synthesis does not appear to be facilitated by sucrose synthase. Instead sucrose synthase may degrade sucrose and yield UDP glucose for starch synthesis (Turner and Turner 1957; Neufeld and Hassid 1963; Bucke and Coombs 1974; Jenner 1980; Liu and Shannon 1981). Cobb and Hannah (1988) have shown that sh1 kernels grown on reducing sugars contain sucrose; this is evidence that sucrose synthase is not necessary for sucrose synthesis and that this enzyme has a role in sucrose degradation instead.

Plant stress such as anaerobiosis, chilling, and wounding have been shown to alter sucrose synthase gene expression as has sucrose concentration. Springer et al. (1986) called the maize Sh1 sucrose synthase an anaerobic protein; however, McElfresh and Chourey (1988) showed that Sh1 mRNA is induced by anaerobiosis but the translation of that message is not induced. Potato sucrose synthase mRNA is also induced by anaerobiosis (Salanoubat and Belliard 1989). Wheat sucrose synthase activity increases after chilling shock while sucrose phosphate synthetase and invertase activity were not altered (Calderon and Pontis 1985). Wounding in potato resulted in a decrease in sucrose synthase mRNA levels while increased sucrose concentrations led to an increase in sucrose synthase mRNA (Salanoubat and Belliard 1989). Increased sucrose

synthase activity in cotton leaves corresponds to increased sucrose concentrations (Hendrix and Huber 1986).

Sucrose synthase is a tetramer; the subunits are generally around 90,000 molecular weight (mol wt). The maize Sh1 sucrose synthase subunit is 88,000 mol wt (Su and Preiss 1978). The maize Sus sucrose synthase subunit is the same size as the Sh1 sucrose synthase subunit and these proteins were given the size of 87,000 mol wt by Echt and Chourey (1985). The sucrose synthase proteins from maize were determined to be 360,000 mol wt (Tsai 1974). Sucrose synthase isolated from wheat germ is 370,000 mol wt while sucrose synthase isolated from wheat leaf tissue is 380,000 mol wt (Larsen et al. 1985). Soybean nodules contain a sucrose synthase of 400,000 mol wt with a subunit mol wt of 90,000 (Morell and Copeland 1985). The sucrose synthase found in peach fruit is 360,000 mol wt, made up of identical subunits of 87,000 mol wt (Moriguchi and Yamaki 1988). Mung bean seedlings have a sucrose synthase of 380,000 mol wt and subunits of 94,000 (Delmer 1972). Rice seeds have a sucrose synthase of 410,000 mol wt and a subunit size of 100,000 mol wt (Nomura and Akazawa 1973).

There are only a few examples of sucrose synthase genes or cDNAs which have been sequenced. Maize is the biological system most explored not only in sucrose synthase protein but also for sucrose synthase structural genes and cDNAs. The sucrose synthase gene of maize, Sh1, has been sequenced as well as the cDNA encoded in that gene. Hence the gene

structure of Sh1 was determined (Werr et al. 1985; Sheldon et al. 1983). Sh1 has 16 exons with the first and second introns being 1014 bp and 511 bp respectively. Based upon RNA analysis the Sh1 gene is predominantly expressed in the endosperm as compared to other tissues (Springer et al. 1985). The Sh1 sequences are the only published sucrose synthase genomic sequences (Geiser et al. 1982; Sheldon et al. 1983; Werr et al. 1985; Zack et al. 1986; Baier and Hannah unpublished). However, a maize Sus sucrose synthase genomic clone has been partially sequenced along with a cDNA clone encoded by that structural gene (McCarty et al. 1986; Shaw and Hannah unpublished). The Sus gene has only 15 exons; when compared to the Sh1 gene structure intron 15 is not present. This sucrose synthase gene, Sus, is largely constitutively expressed (Springer et al. 1985; McCarty et al. 1986; Hannah and McCarty 1988).

Besides the two maize sucrose synthase cDNAs a potato sucrose synthase cDNA has also been sequenced. This potato cDNA includes the entire protein coding region (Salanoubat and Belliard 1987). Another dicot sucrose synthase cDNA, from soybean, has been partially sequenced; this 500 bp cDNA has similarity with exons 14, 15, and 16 of the maize Sh1 gene (Thummler and Verma 1987). Two wheat sucrose synthase cDNAs have been partially sequenced (Marana et al. 1988). These two wheat cDNAs including most of exon 13, and exons 14, 15, and 16 were compared to the maize Sh1 sequence. One of the wheat

sucrose synthase cDNAs was more similar to the Sh1 sequence than to the other wheat cDNA sequence.

In the work presented here, Pennisetum purpureum sucrose synthase and the sucrose synthase subunits are shown to be similar in size to the maize counterparts. Two putative P. purpureum sucrose synthase genomic sequences were identified and cloned; one of these genomic clones was partially sequenced and compared to the Sh1 and Sus gene structures. Two nearly full length cDNA clones were selected from a leaf cDNA library. One of these which is coded for in the partially sequenced genomic clone was sequenced. The sequenced sucrose synthase genomic and cDNA clones from P. purpureum are more homologous to the Sus gene from maize than to the Sh1 gene; the other sucrose synthase cDNA appears to have equal similarity to either of the maize sucrose synthase genes based upon cross hybridization. An additional gene from P. purpureum was cloned. It has similarity with the 5' end of the Sh1 gene from maize. This unidentified genomic clone may code for an abundant 1.9 kb mRNA which hybridizes to the 5' end of sucrose synthase structural genes. Also, a P. glaucum sucrose synthase cDNA of 2.5 kb was sequenced and shown to have more similarity to the Sus gene of maize than to the Sh1 gene. The sequenced P. purpureum sucrose synthase cDNA clone is compared to the P. glaucum sucrose synthase cDNA as well as to the maize sucrose synthase cDNA from the Sus locus.

CHAPTER 2
MATERIALS AND METHODS

DNA Isolation, RNA Isolation, Digestion, and Fractionation

All Pennisetum species were supplied by Dr. W.W. Hanna (Coastal Plain Exp. Station, Tifton, Georgia) except for P. purpureum (PI300086) which came from Dr. S.C. Schank (University of Florida). DNA was isolated from mature leaf tissue of plants (Rivin et al. 1982). Approximately 10 g to 20 g of leaf tissue was used. The Kpn I monomers used as probe DNA and for the copy number determination were isolated from 3% polyacrylamide gels by diffussion into TE [10 mM Tris-Cl (pH 7.8) and 1 mM EDTA (ethylenediamine-tetracetic acid)] over night at 4^0 C. Insert DNA from recombinant plasmids was isolated from agarose gels by electrophoresis into wells containing gel buffer and 10% glycerol (Maniatis et al. 1981).

Nuclear DNA was isolated from mature leaf tissue following an unpublished procedure from Dr. Eduardo Vallejos. Twenty grams of tissue was cut into small pieces with scissors and immersed into five volumes of ice cold 100 mM Tris-HCl (pH 8.0), 10 mM EDTA, 500 mM sucrose, and 0.1% mercaptoethanol. The leaf tissue was homogenized in a Waring blender for 60 seconds. The homogenate was filtered through cheese cloth.

Triton X-100 was added to a final concentration of 0.5%. The samples were spun at 2000 rpm for 15 mins at 4^0 C. The pellet was resuspended at 1 ml/10 g of tissue in 100 mM Tris-HCl (pH 8.0) and 20 mM EDTA. An equal volume of the above solution with 100 ug/ml of lysozyme was added. This suspension was mixed then incubated at 65^0 C for 30 mins with occasional inversion. After incubation the suspension was extracted once with phenol:chloroform:isoamyl alcohol (50:48:2) and centrifuged at 5,000 rpm. The supernatant was reextracted with chloroform:isoamyl alcohol (96:4) and centrifuged. The DNA in the supernatant was then cesium chloride purified (Maniatis et al. 1982).

Plasmid minipreparations followed the procedure below. Fifty ml cultures of cells were grown overnight in LB medium with 100 ug per ml of ampicillin. The cells were chilled, centrifuged, and resuspended in 580 ul of 25% sucrose and 50 mM Tris-HCl (pH 7.8). Oak Ridge tubes were kept on ice through the following steps up to the extractions. A 100 mg/ml lysozyme solution was made with the above sucrose solution and 200 ul was added to the cell suspension. After five minutes 475 ul of 500 mM EDTA was added. After five minutes 1.25 ml of 0.1% Triton X-100, 50 mM Tris-HCl (pH 7.8), and 10 mM EDTA was added. After 20 minutes the cell debris was centrifuged out at 15,000 rpm for 30 mins. The supernatent was extracted with phenol:chloroform:isoamyl alcohol and centifuged at 5,000 rpm for 5 mins, then reextracted with chloroform isoamyl alcohol.

The supernatant was precipitated with 1/10 volume 3 M sodium acetate and two volumes ethanol. The precipitant was centrifuged at 10,000 rpm for 15 mins. The pellet was resuspended in TE and reprecipitated with 0.5 volume of 7.5 M ammonium acetate and two volumes of ethanol. The precipitant was pelleted, washed with 70% ethanol, dried, and resuspended in TE.

Lambda DNA minipreparations involved scraping off the top agarose from two petri plates exhibiting confluent lysis. The top agarose was shaken at 100 rpm at room temperature in 25 ml of SM [50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 8 mM $MgSO_4$, and 0.01% gelatin] for three hours. At this point a standard procedure was followed (Maniatis et al. 1982).

RNA was isolated from P. purpureum mature leaf tissue, P. glaucum immature seed, and maize kernel using procedure for maize (McCarty 1986).

DNA digestions for genomic, pUC 19, and M13 mp19 samples were carried out according to the suppliers procedure. Restriction endonucleases were from Bethesda Research Laboratories (BRL) except Bst NI which came from New England Biolabs.

DNAs were fractionated in 3% polyacrylamide along with 123 bp markers (BRL). A Bio-Rad 16 cm gel apparatus was used. Gel widths were 1.5 cm. The buffer system employed was TBE (100 mM Tris base, 100 mM Boric acid, and 20 mM EDTA). The 3% polyacrylamide gels contained 0.1% bis-acrylamide, 0.0005%

TEMED, and 0.12% ammonium persulfate. Samples were electrophoresed at 80 volts from 5 to 7 hours. After electrophoresis, the DNA was stained with ethidium bromide (5 ug/ml) for 15 minutes. The gels were illuminated with an Ultra-violet Products, Inc. transilluminator and photographed with a Polaroid MP-4 Land Camera.

DNAs were fractionated in 0.8% to 1.25% agarose. BRL H4 and H5 gel electrophoresis units were used with TBE buffer. Samples were electrophoresed at varying voltage depending on the length of the run and the sample migration distance. After electrophoresis the gels were stained and photographed as above.

RNAs were fractionated in 1.2% agarose/formaldehyde gels at 50 volts using a BRL H4 electrophoresis unit. The MOPS buffer and procedure is described in Maniatis et al. (1982).

Southern Blotting, RNA Blotting, Probe Labeling, and Hybridizations

DNA was blotted onto nylon membrane (Amersham Hybond N or Hybond N+) following Maniatis' procedure (Maniatis et al. 1982) except the blotting was performed on foam rubber in a tray instead of the wick method. When Hybond N was used, the transfer buffer was 20X SSPE [3.6 M NaCl, 200 mM NH_2PO_4 (pH 7.7), and 20 mM EDTA]. After blotting onto Hybond N the DNA was crosslinked to the nylon by 8 minutes of UV irradiation.

When the DNA was blotted onto Hybond N+ the transfer solution was 400 mM NaOH.

RNA was blotted onto Amersham Hybond. After electrophoresis the gels were soaked in 20X SSPE for 10 minutes and then blotted identically to a Southern blot using 20X SSPE. This was followed by crosslinking and hybridization.

Two types of hybridization procedures were used. In the first, the prehybridization solution was 5X Denhardt's, 5X SSPE, 0.1% SDS (sodium lauryl sulfate), and 150 ug/ml of denatured and sheared salmon sperm DNA. The hybridization solution was 2X Denhardt's, 5X SSPE, 0.1% SDS, and 75ug/ml salmon sperm DNA (Maniatis et al. 1982). In the second hybridization procedure, the prehybridization and hybridization solutions were the same: 500 mM Na_2HPO_4 (pH 7.2), 1% BSA (bovine serum albumin), and 7% SDS (Church and Gilbert 1984). For both procedures prehybridization was approximately one hour and hybridization extended overnight. The temperatures for the above procedures were from 60^0 C to 68^0 C as indicated in the figure legends. Probe labeling involved a nick translation kit or a random primer kit (BRL) following the suppliers procedure. Hybridizations were carried out in a hot air incubator with rotation. Washing conditions included a five minute wash at room temperature in 2X SSPE and 0.1% SDS followed by at least two washes in 0.07X SSPE or 0.1X SSPE and 0.1 % SDS as indicated in the figure legends. Washing

temperatures were at the same temperatures as the hybridizations.

After washing, the filters were dried. The distribution of alpha [^{32}P]-labeled DNA on the blots was determined by autoradiography using Kodak X-OMAT AR film and intensifying screens at -70^{0} C for various lengths of time. The autoradiographs were developed using a Konica QX-60A developer.

Genomic and cDNA Cloning

Two genomic libraries and two cDNA libraries were constructed. The first genomic library utilized the vector lambda gtwes; the insert DNA was 5 kb to 10 kb Eco RI P. purpureum (PI300086) DNA. Both vector arms and insert DNA were isolated from sucrose gradients and cloned using standard techniques (Maniatis et al. 1982). Promega Corporation Packagene Extract was used to package the ligation products. Genomic clones were plated and screened using Amersham nitrocellulose (Maniatis et al. 1982). The second genomic library was constructed with 6 kb to 11 kb Bam HI P. purpureum (PI300086) DNA isolated from an agarose gel and cloned into EMBL3 prepared according to the suppliers procedure (Promega Corporation). The same packaging, plating, and screening procedures were followed except Amersham Hybond N^{+} was used. To construct the two cDNA libraries a BRL cDNA

synthesis kit was used following the suppliers directions. The same packaging, plating, and screening procedures were followed with Amersham Hybond N^+.

Sucrose Synthase Isolation and Antibody Production

Sucrose synthase was isolated by Dr. D.R. McCarty (Echt and Chourey 1985) for use in antibody production. Isolated protein was used in eliciting an antibody response in a rabbit. Anti-Sh1 antibody staining followed a procedure by Bio-Rad.

Copy Number Determination

The percentage of the genomes composed of the Kpn I repetitive sequence families was based upon hybridization of Kpn I monomers to 100 nanograms (ng) of nuclear DNA and standard amounts of isolated Kpn I monomers (5, 10, 15, 20, 25, and 30 ng). A dot blot apparatus was used (Schliester and Schull). The experiments were replicated three times. The P. purpureum (PI 300086) dot blots contained a combination of 140 bp and 160 bp Kpn I monomers as standards to compare with 100 ng of nuclear DNA; the probe DNA was the same as the DNA used as the standards. In determining the copy number of the Kpn I tandemly arrayed repetitive sequence families in P. purpureum the size of the repetitive element used in the calculation was

150 bp. The P. glaucum dot blots were composed of genotype Tift 18DB nuclear DNA compared to genotype Tift 23 Kpn I monomers; the probe DNA was the same monomers as the standards. After hybridization of the probes to the dot blots, washing, and autoradiography the radioactive areas of the dot blots were counted in a scintillation counter. Each of the three experiments for both sets of samples was treated separately. A standard curve was drawn for each experiment and the percentage of the genome hybridizing to the probe was determined from the curve. An average value with a standard deviation was calculated for both sets of experiments. The average value was used to determine the copy number.

To determine the copy number from the percentage of the genome(s) composed of these repeats the following procedure was used. The average weight of a nucleotide pair is 618 g/mole. In one picogram there are 1.6181×10^{-15} moles. When the number of moles in one picogram is multiplied by Avagodro's number (6.022×10^{23} nucleotide pairs/mole) the value for the number of base pairs in one picogram is 9.7443×10^{8}. This value of base pairs per picogram was used to determine the size of the genome(s) in base pairs from the published sizes of the P. purpureum and P. glaucum genomes which were given in picograms. The size of the genome(s) in base pairs was multiplied by the percentage of the genome(s) which hybridized the Kpn I probes to identify the number of base pairs which hybridized the probe. This figure was then

divided by the size of the Kpn I monomers to determine the copy number per cell of these tandemly arrayed repetitive sequences.

DNA Sequencing

The Kpn I repetitive sequence monomers from P. purprueum, P. glaucum, P. squamulatum, and P. hohenackeri were sequenced with the Singer dideoxy method (Sanger et al. 1977). These monomers were originally cloned into pUC19 for sizing and then subcloned into M13mp19. Then M13mp19 subclones were either sequenced in both directions or sequenced twice by John Baier. An IBI Gel Reader was utilized with the subsequent sequences analyzed with IBI DNA/Protein Sequence Analysis software. The Kpn I monomers from P. setaceum and P. villosum were sequenced in both directions directly in pUC19 by Dr. Ernesto Almira at the ICBR DNA Sequencing Core Facility using a Genesis 2000 DNA Analysis System. This method involved fluorescent chain-terminating dideoxynucleotides (Prober et al. 1987).

The two sucrose synthase cDNA clones, pc309 and pcPMSS, were originally subcloned into pUC19. These Eco RI inserts were then subcloned into pGEM-7Zf(+) from Promega Corporation. Deletions of these two clones proceeded in either direction using the Erase-a-Base System from Promega. Deletions in the T7 direction were performed with both cDNA clones being double digested with Aat II (3' overhang for inhibition of

exonuclease III) and Xho I. Deletions in the SP6 direction involved the enzymes Nsi I (3' overhang) and Bam HI. Deletions of these clones were selected from minipreped plasmid DNA and sequenced at the ICBR sequencing core in collaboration with Dr. Ernesto Almira. The gaps in the sequencing data for the two clones were filled by using 20 base oligonucleotides as primers to sequence through areas where deleted subclones were not obtained. The individual strands for the two clones were compared using IBI DNA/Protein Analysis software. Approximately 98% of the bases in the two strands aligned. The most common ambiguous bases resulted from C/T and A/G mismatches. A common location for ambiguous bases was when an A or G followed a T which gave a strong signal. The corrected sequences of pc309 and pcPMSS were compared; bases not aligning between the two sucrose synthase cDNAs were further reviewed. Additional sequence comparison between the Pennisetum clones and maize sucrose synthase cDNAs utilized the above IBI software.

Isozyme Electrophoresis and Staining

Starch gel electrophoresis, as described by Shields et al. (1983), was employed to fractionate the isozymes. Protein extraction was performed with leaf tissue frozen in liquid N_2, the buffer was 0.1M Tris-Cl (pH 7.8), 10mM DTT (dithiothreitol) and 20% glycerol. The ratio of leaf weight to

buffer was 1 gm per ml. Wicks saturated with extract were stored at -70^0 C. Two buffer systems were used for gels and electrodes. The first involves citric acid as the electrode buffer and histidine as the gel buffer (Flides and Harris 1966); MDH (malate dehydrogenase) was the only isozyme stained for with this buffer system. The second buffer system was used for PGI (phosphoglucoisomerase), APS (alkaline phosphatase), GOT (glutamate oxaloacetate transaminase), and ADH (alcohol dehydrogenase). This buffer system included boric acid as the electrode buffer and tris citrate as the gel buffer (Ridgway et al. 1970). Staining for MDH, PGI, APS, and ADH was performed as in Vallejos (1983). Staining for GOT is from Bournival et al. (1989).

CHAPTER 3
RESULTS OF PROGENITOR STUDY

Total DNAs from six P. purpureum genotypes were digested with Kpn I, electrophoresed in 3% polyacrylamide, and stained with ethidium bromide (Fig. 1). All six genotypes contain 140 bp and 160 bp Kpn I families of tandemly arrayed repetitive sequences indicating a species wide pattern. P. purpureum genotypes N-16 (lane 3) and Merkeron (lane 4) exhibit a greater proportion of 160 bp Kpn I monomers compared to 140 bp Kpn I monomers. P. purpureum genotype PI300086 has similar amounts of 160 bp and 140 bp Kpn I monomers (lane 2).

To compare the allotetraploid P. purpureum to additional Pennisetum species (Fig. 2A), total DNAs from P. hohenackeri (lane 2), P. squamulatum (lane 3), P. purpureum (lane 4), and P. glaucum (lane 5) were digested with Kpn I and electrophoresed as above. P. squamulatum and P. purpureum share a common digestion pattern, both containing 140 bp and 160 bp repetitive sequence families. The diploid pearl millet contains a 140 bp Kpn I family while the diploid P. hohenackeri contains a 160 bp family. P. hohenackeri is not considered to be a closely related species of P. purpureum based upon phenotype, isozyme banding patterns (Lagudah and Hanna 1989), and additional evidence presented in this paper.

Fig. 1. Restriction endonuclease digestion pattern of *P. purpureum* DNAs. *P. purpureum* total DNAs (7.5 ug) were digested with *Kpn* I, electrophoresed in 3% polyacrylamide, and stained with ethidium bromide. The 140 bp and 160 bp *Kpn* I family monomers are labeled. Lane 1 is 123 bp markers. *P. purpureum* genotypes are PI300086 (lane 2), N16 (lane 3), Merkeron (lane 4), N166 (lane 5), N137 (lane 6), and N138 (lane 7).

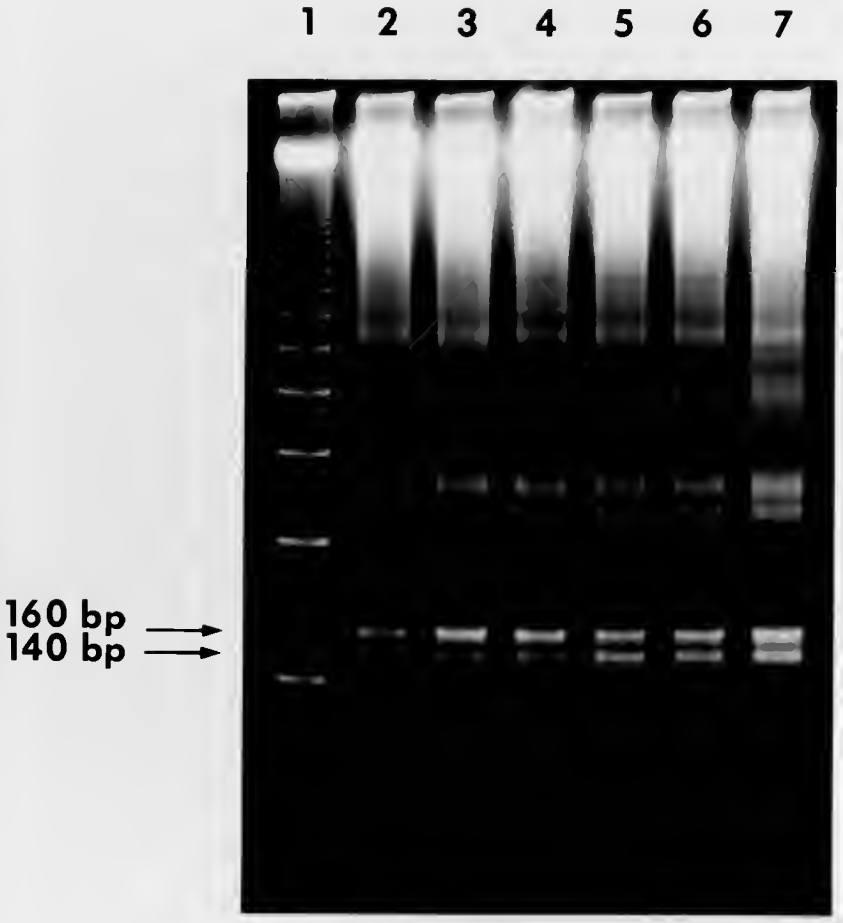
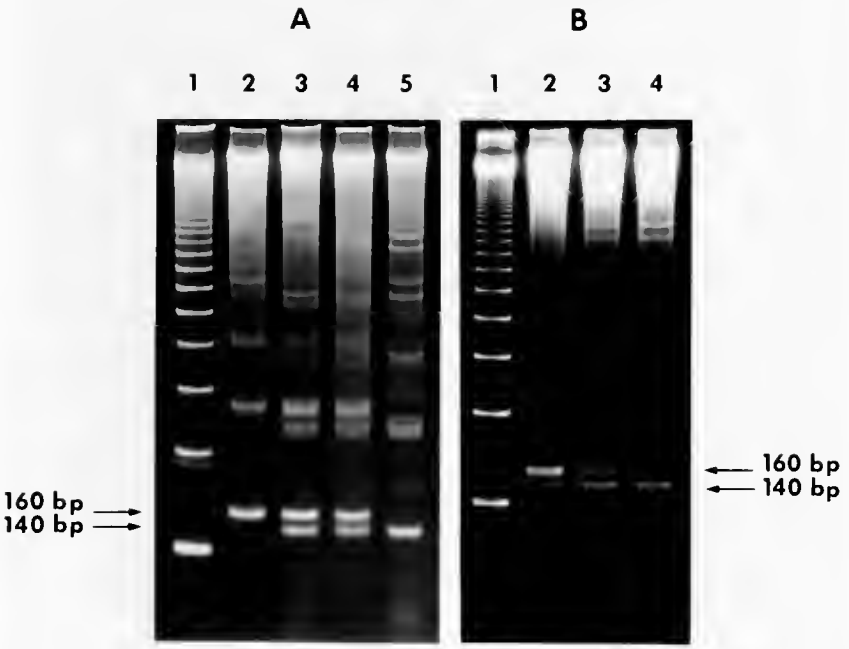

1 2 3 4 5 6 7
160 bp
140 bp

Fig. 2. Restriction endonuclease digestion pattern of Pennisetum DNAs. Total DNAs (7.5 ug) from selected Pennisetum species were digested with Kpn I, electrophoresed in 3% polyacrylamide, and stained with ethidium bromide. The 140 bp and 160 bp Kpn I family monomers are labeled. For A, lane 1 is 123 bp markers. Total DNAs are P. hohenackeri (PS156) (lane 2), P. squamulatum (PS26) (lane 3), P. purpureum (PI300086) (lane 4), and P. glaucum Tift 23 (lane 5). For B, lane 1 is 123 bp markers. Total DNAs are P. purpureum (N16) (lane 2), P. purpureum (N16) X P. glaucum Tift 23A (lane 3), and P. glaucum Tift 23A (lane 4).


A
B
1 2 3 4 5
1 2 3 4
160 bp
140 bp
160 bp
140 bp

However, the appearance of the 160 bp Kpn I family of P. hohenackeri indicated further investigation was necessary in identifying the relationship between the 160 bp Kpn I families of the species depicted in Fig. 2A.

Fig. 2B displays the Kpn I digestion pattern of P. purpureum (N16) (lane 2), P. glaucum Tift 23A X P. purpureum (N16) (lane 3), and P. glaucum Tift 23A (lane 4). In P. purpureum half of the chromosomes belong to the A' genome while the other half belong to the B genome. The proposed progenitor of P. purpureum, P. glaucum (A genome designation), has a 140 bp Kpn I family; this suggests the 140 bp Kpn I family of P. purpureum is located within the A' genome. Hence the 160 bp Kpn I family of P. purpureum may be a marker for the B genome. This experiment addressed the question of the Kpn I families being genome markers in P. purpureum. The triploid interspecific hybrid has the genome designation of AA'B; the A complement from P. glaucum and the A'B complement from P. purpureum. The Kpn I digest of the triploid shows a combination of bands present in both parents. There is a redistribution of 140 bp and 160 bp Kpn I families as seen by ethidium bromide staining intensity of the triploid as compared to P. purpureum. The P. purpureum parent has a predominance of the 160 bp Kpn I monomer over the 140 bp Kpn I monomer. The triploid has a predominance of the 140 bp Kpn I families as would be expected since 14 of the 21 chromosomes in the triploid hybrid are either A or A' from P. glaucum or

P. purpureum respectively. This redistribution pattern of Kpn I families indicates that the 140 bp Kpn I family of P. purpureum may be an A' genome marker while the 160 bp Kpn I family may be a B genome marker.

To investigate cross hybridization of these Kpn I families DNA from the Pennisetum species in Fig. 2A were digested, electrophoresed, blotted, and probed with nick translated 140 bp Kpn I sequences from P. glaucum (Fig. 3). Total DNAs from P. hohenackeri (lane 1), P. squamulatum (lane 2), P. purpureum (lane 3), and P. glaucum (lane 4) and nuclear DNAs from P. purpureum (lane 5) and P. glaucum (lane 6) were probed. Probing of nuclear DNA showed that the Kpn I families are of nuclear origin. Stringent washing conditions were used indicating a strong similarity between these families of tandemly arrayed repetitive sequences. The 140 bp Kpn I monomers from P. glaucum used as a probe cross hybridized with the other 140 bp Kpn I families and also with the 160 bp Kpn I families. This cross hybridization suggested that these sequences have a common origin.

Fig. 4 shows Kpn I families of additional genotypes of species already observed and also additional Pennisetum species. Total DNAs were digested, electrophoresed in 3% polyacrylamide, and stained with ethidium bromide. Lane 1 is a second P. squamulatum genotype, PS24; this genotype compares with P. squamulatum genotype PS26 seen in Fig. 2A except there is less DNA hence the repeats are not as visible. Lane 2 and

Fig. 3. Cross hybridization of the Kpn I families from the Pennisetum species shown in Fig. 2. Total DNAs (2 ug) from Pennisetum species were digested with Kpn I, electrophoresed in 1.75% agarose, Southern blotted, probed at 65^0 C with nick translated pearl millet IB23 140 bp Kpn I monomers,and washed at 65^0 C in 0.07X SSPE and 0.1% SDS. Concatomers of three repeats from the 140 bp and 160 bp Kpn I families are labeled as 420 bp and 480 bp respectively. Total DNAs are P. hohenackeri (PS156) (lane 1), P. squamulatum (PS26), (lane 2), P. purpureum (PI300086) (lane 3), and P. glaucum Tift 23 (lane 4). Nuclear DNAs are P. purpureum (PI300086) (lane 5) and P. glaucum Tift 18DB (lane 6).

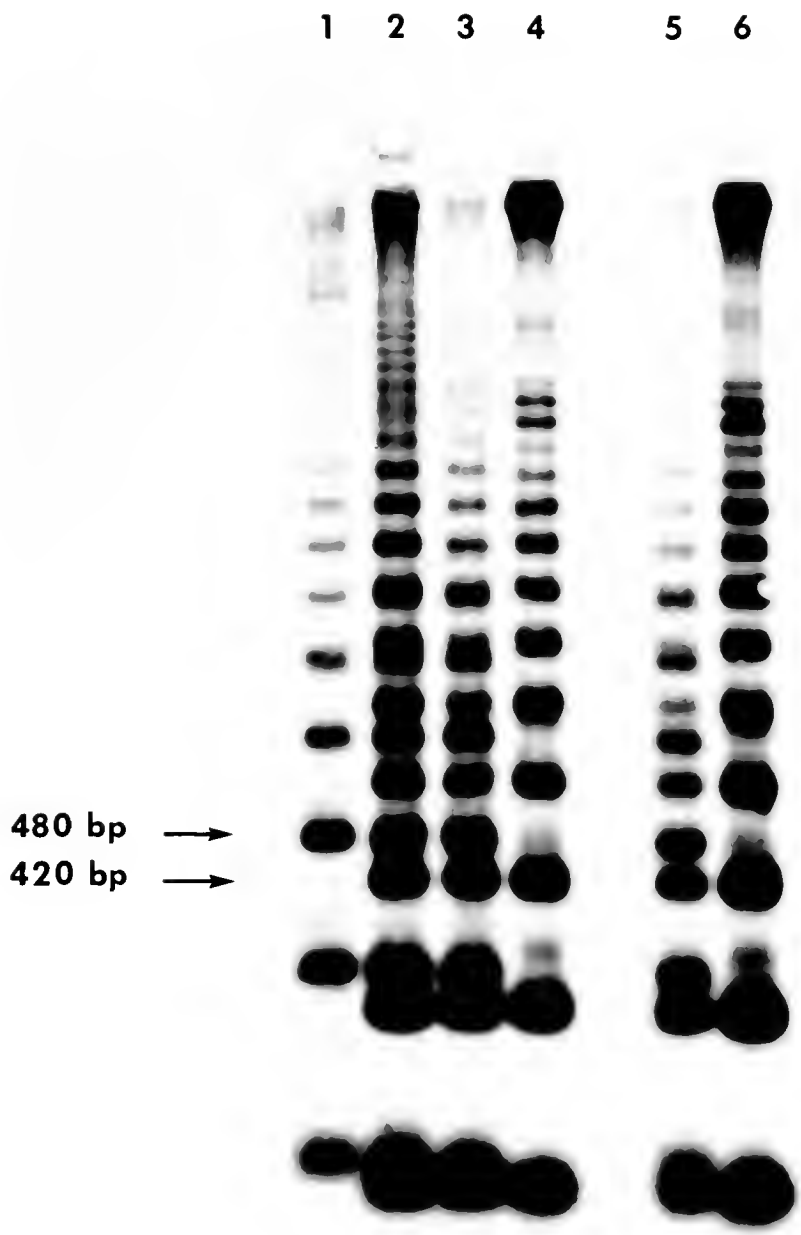
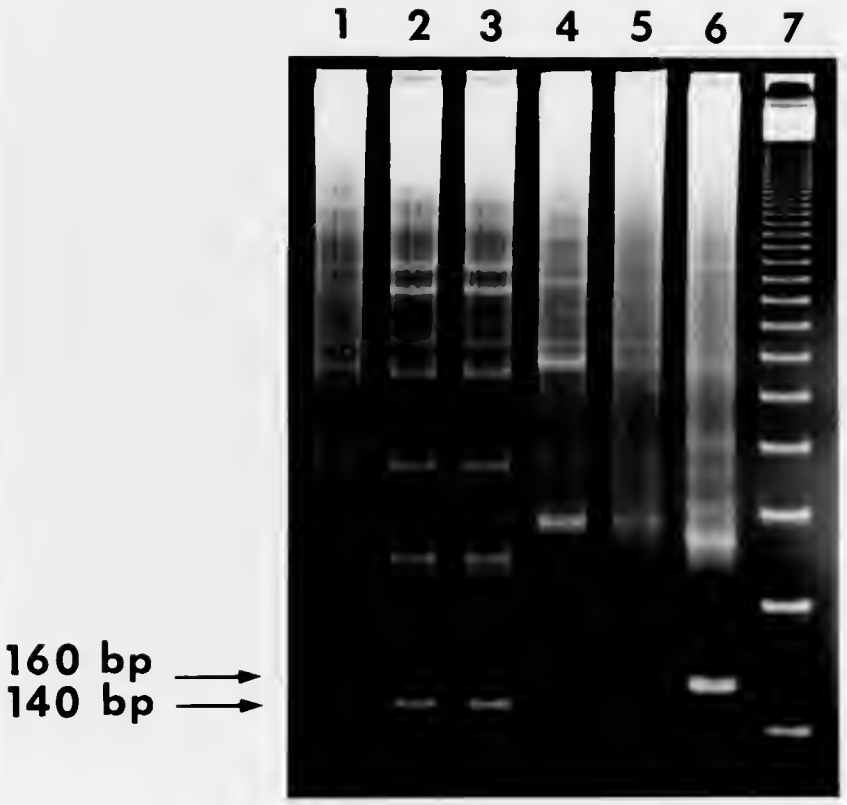

1
2
3
4
5
6
480 bp
420 bp

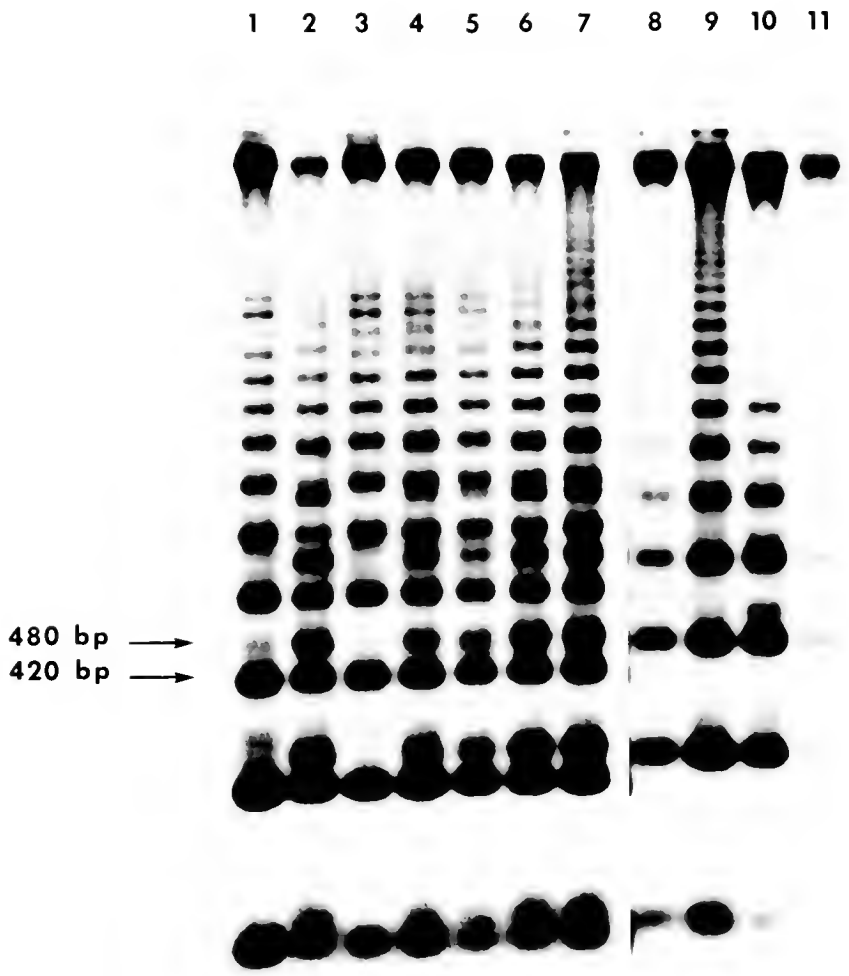
Fig. 4. Restriction endonuclease digestion pattern of additional Pennisetum DNAs. Total DNAs were digested with Kpn I, electrophoresed in 3% polyacrylamide, and stained with ethidium bromide. All samples were 7.5 ug except P. squamulatum which was 2.5 ug. The 140 bp and 160 bp Kpn I family monomers are labeled. Total DNAs are P. squamulatum (PS24) (lane 1), P. glaucum ssp. monodii (PS470) (lane 2), P. glaucum ssp. monodii (PS34) (lane3), P. setaceum (PS49) (lane 4), P. setaceum (PS247) (lane 5), and P. villosum (PS249) (lane 6). Lane 7 is 123 bp markers.


1 2 3 4 5 6 7
160 bp
140 bp

3 are two genotypes of P. glaucum ssp. monodii. These are similar in digestion pattern to the pearl millet genotypes seen in Fig. 2. In lanes 4 and 5 are two genotypes of P. setaceum (Forsk.) Chiov. P. setaceum, a polymorphic species often characterized as a triploid, also has a 160 bp Kpn I family of repeated DNA. P. villosum R. Br. ex Fresen. is seen in lane 6, this polymorphic species has an intensely staining 160 bp Kpn I family present in the digest. P. villosum pachytene chromosomes when stained with acetocarmine or aceto-orcein exhibit terminal heterochromatic knobs and centromeric bands (Juahar 1981). These large heterochromatin regions may contain the intensely staining 160 bp Kpn I band seen in lane 6.

In Fig. 5 total DNA from 11 Pennisetum species and interspecific hybrids was digested with Kpn I, electrophoresed, Southern blotted, and probed with 140 bp Kpn sequences from P. glaucum. P. glaucum ssp. monodii is in lane 1; no difference has been observed between the repetitive sequence families of P. glaucum ssp. monodii and pearl millet as shown by hybridization or electrophoresis in 3% polyacrylamide, including the appearance of a 170 bp Kpn I family visible only by hybridization. Lanes 2-4 are the genotypes seen in Fig. 2B, P. purpureum (N16), P. glaucum Tift 23A, and the P. glaucum X P. purpureum triploid respectively. A redistribution of the Kpn I families at the 420 bp and 480 bp positions in the triploid when compared to the P. purpureum

Fig. 5. Cross hybridization of the Kpn I families of additional Pennisetum species and interspecific hybrids. Total DNAs from Pennisetum species and interspecific hybrids were digested with Kpn I, electrophoresed in 1.75% agarose, Southern blotted, probed at 65^0 C with random primer labeled pearl millet IB23 140 bp Kpn I monomers, and washed at 65^0 C in 0.07X SSPE and 0.1% SDS. All samples were 2 ug except P. orientale which was 6 ug. Concatomers of three repeats from the 140 bp and 160 bp Kpn I families are labeled 420 bp and 480 bp respectively. Total DNAs are P. glaucum ssp. monodii (PS34) (lane 1), P. purpureum (N16) (lane 2), P. glaucum Tift 23A (lane 3), P. glaucum Tift 23A X P. purpureum (N16) (lane 4), P. glaucum (H165) X P. squamulatum (PS262) (lane 5), P. purpureum Merkeron X P. squamulatum (PS262) (lane 6), P. squamulatum (PS26) (lane 7), P. setaceum (PS247) (lane 8), P. villosum (PS249) (lane 9), P. orietale (PS15) (lane 10), and P. flaccidum (PS396) (lane 11).


1 2 3 4 5 6 7 8 9 10 11
480 bp
420 bp

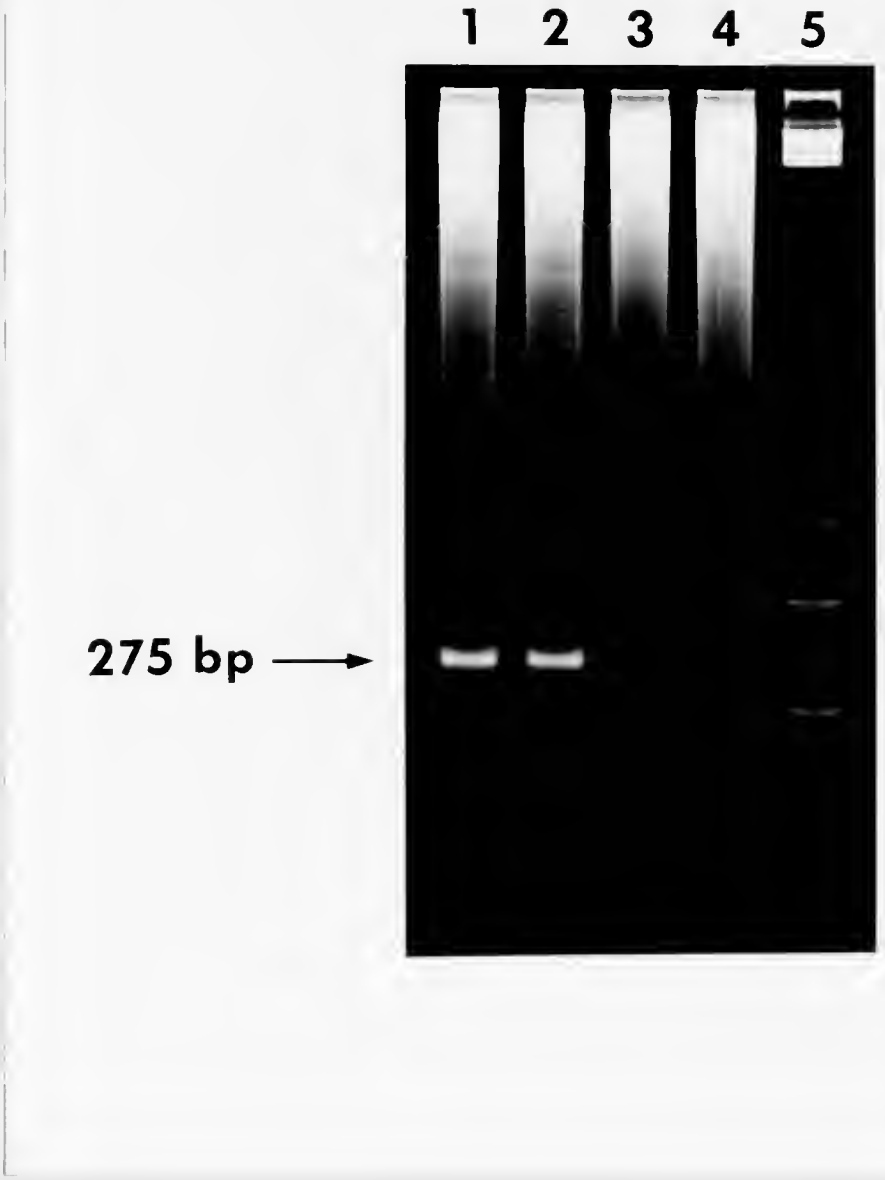
The triploid in lane 4 has more hybridization of the probe at 420 bp versus 480 bp as expected because there are twice as many A\A' chromosomes as B chromosomes. A P. glaucum (H165) X P. squamulatum (PS262) hybrid is shown in lane 5. The additional A chromosomes from P. glaucum have combined with the chromosomes from P. squamulatum to increase the amount of the 140 bp Kpn I family as compared to the 160 bp Kpn I family; this is indicated by the hybridization intensity of the probe at the 420 bp region versus the 480 bp region. Lane 6 is an interspecific hybrid between P. purpureum Merkeron X P. squamulatum (PS262). P. squamulatum (PS26) is in lane 7. The Southern blot resulting in the autoradiograph in Fig. 5 was exposed for two periods of time to observe comparable amounts of hybridization between lanes 1-7 and lanes 8-11. Lanes 8-11 represent a longer exposure. P. setaceum (PS247) is shown in lane 8. Cross hybridization between the pearl millet probe and P. setaceum as well as the rest of the Pennisetum species indicates similarity between these Kpn I families. P. villosum (PS249) was characterized in Fig 4 as having an intensely staining 160 bp Kpn I family; in lane 9 this Pennisetum has strong hybridization signals further suggesting a large proportion of the P. villosum genome is comprised of this repeat family. The quantity of P. orientale L.C.Rich. DNA in lane 10 is three times the amount of DNA in the other lanes; hence, there is less of the 160 bp Kpn I family than is indicated by the hybridizational intensity. Also present in P.

orientale is a tandem repeat slightly larger than the band seen at 480 bp. Lane 11 is P. flaccidum Griseb., the bands of hybridization are barely visible.

The appearance of the 160 bp Kpn I family of tandemly arrayed repetitive sequences in most of the Pennisetum species characterized here indicates that this repetitive sequence family is common within the genus. Of all the Pennisetum species observed, only P. glaucum (pearl millet and ssp. monodii) does not have a detectable 160 bp Kpn I family. The 140 bp Kpn I family is present in the closely related species P. purpureum, P. squamulatum, and P. glaucum (including pearl millet and ssp. monodii). P. glaucum and P. purpureum are in the Pennisetum section Penicillaria while P. squamulatum is in the section Heterostachya (Jauhar 1981). Additional Pennisetum species, especially in the section Penicillaria, may contain 140 bp Kpn I families. Over all, species characterized in this paper represent four of the five sections within the genus Pennisetum; all of these sections have species containing the 160 bp Kpn I family of repeats. Moreover, the species containing these repeat families are from the X=7 and the X=9 groups of the genus.

A Hind III family of repetitive sequences was identified in P. squamulatum. The family is not apparent in P. purpureum (Fig. 6). Hind III was used to digest total DNA from two genotypes each of P. squamulatum and P. purpureum followed by electrophoresis in 3% polyacrylamide and visualization with

Fig. 6. Restriction endonuclease digestion pattern of two *Pennisetum* species. Total DNAs (7.5 ug) were digested with *Hind* III, electrophoresed in 3% polyacrylamide, and stained with ethidium bromide. The 275 bp *Hind* III repeats are labeled. Total DNAs are *P. squamulatum* (PS24) (lane 1), *P. squamulatum* (PS26) (lane 2), *P. purpureum* (PI300086) (lane 3), and *P. purpureum* (N16) (lane 4). Lane 5 is 123 bp markers.


1
2
3
4
5
275 bp

staining. The repeat appears to exist only in monomeric form as indicated by the lack of tandem arrays seen in the fractionated DNA. The presence of this repeat suggests that P. squamulatum contains genetic information not present in P. purpureum. An alternative possibility is that P. purpureum may have this Hind III family in an undigestible form as cryptic DNA.

To further elucidate the tandem arrangement of the Kpn I families, DNAs from P. glaucum (Fig. 7, Lanes 1-3), P. purpureum (lanes 4-6), P. squamulatum (lanes 7-9), and P. hohenackeri (lanes 10-12) were digested for 2, 5, and 60 minutes. The DNAs were then electrophoresed, Southern blotted, and probed with random primer labeled pearl millet 140 bp Kpn I sequences. As digestion proceeds for each sample there was a decrease in the larger concatomers of repeats and an increase in the smaller concatomers. These results demonstrate the tandem repeat arrangement of these families.

In Fig. 8 methylation of the P. purpureum Kpn I families of repeats is demonstrated. P. purpureum DNA was digested with Eco RII (lane 1), then redigested with Eco RII (lane 2) to attempt to completely digest the DNA. In lane 3 P. purpureum DNA was digested with the Eco RII methylation insensitive isoschizimer Bst NI and then redigested (lane 4). These digests were electrophoresed, blotted and probed with random primer labeled pearl millet 140 bp Kpn I sequences. As can be seen, the methylation sensitive Eco RII did not cleave the Kpn

Fig. 7. Incomplete and complete DNA digestions from selected Pennisetum species. Total DNAs were digested for 2 min, 5 min, and 60 min with Kpn I, electrophoresed in 1.25% agarose, Southern blotted, probed at 65^0 C with pearl millet IB23 140 bp Kpn I monomers, and washed at 65^0 C in 0.1X SSPE and 0.1% SDS. All samples were 2 ug; aliquots for each species came from the same reaction mixture. Concatomers of two repeats from the 140 bp and 160 bp Kpn I families are labeled 280 bp and 320 bp respectively. Lanes 1 to 3 are P. glaucum Tift 23 (2 min, 5 min, and 60 min respectively). Lanes 4 to 6 are P. purpureum (PI300086) (2 min, 5 min, and 60 min respectively). Lanes 7 to 9 are P. squamulatum (PS26) (2 min, 5 min, and 60 min respectively). Lanes 10 to 12 are P. hohenackeri (PS156) (2 min, 5 min, and 60 min respectively).

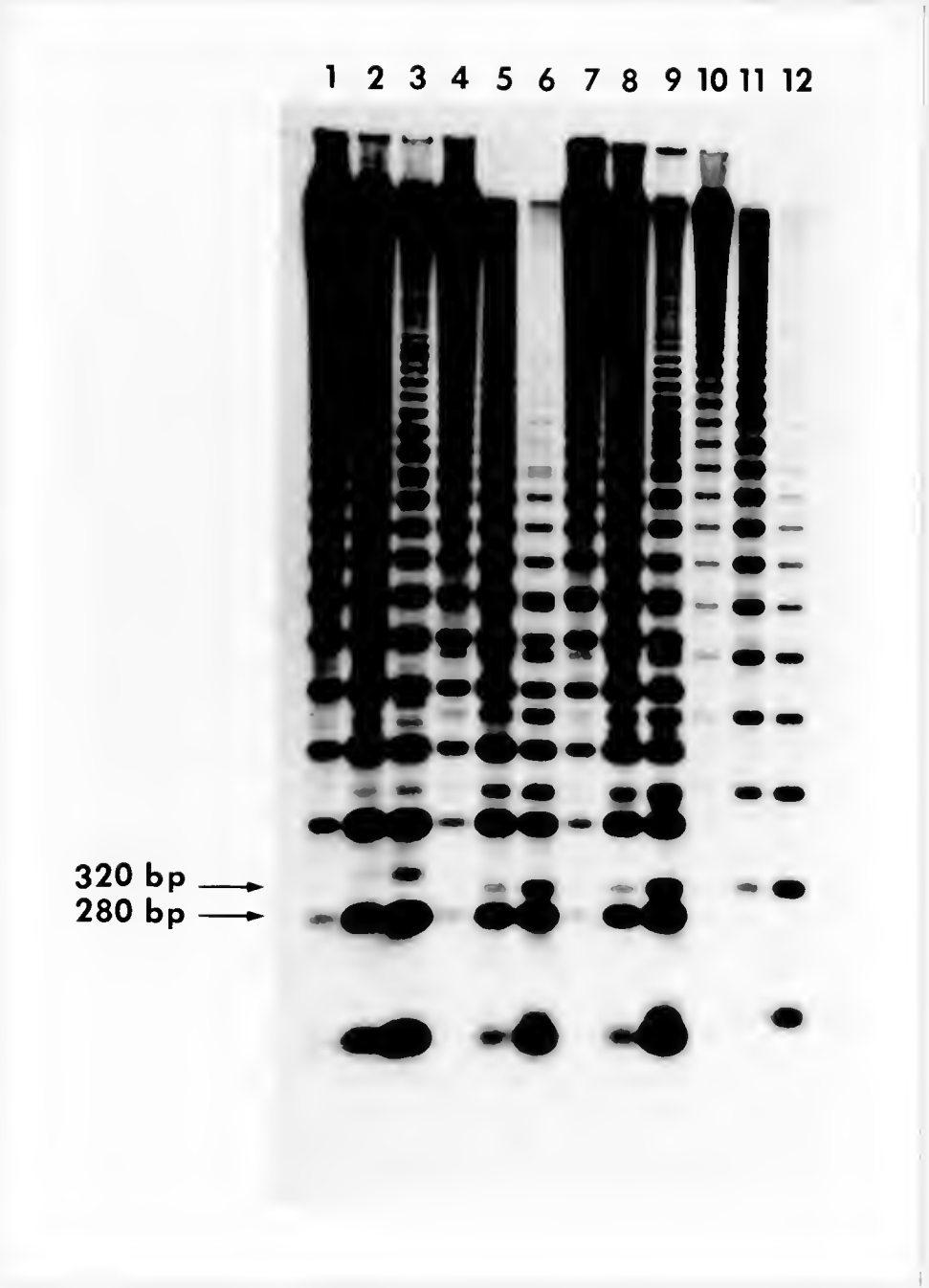
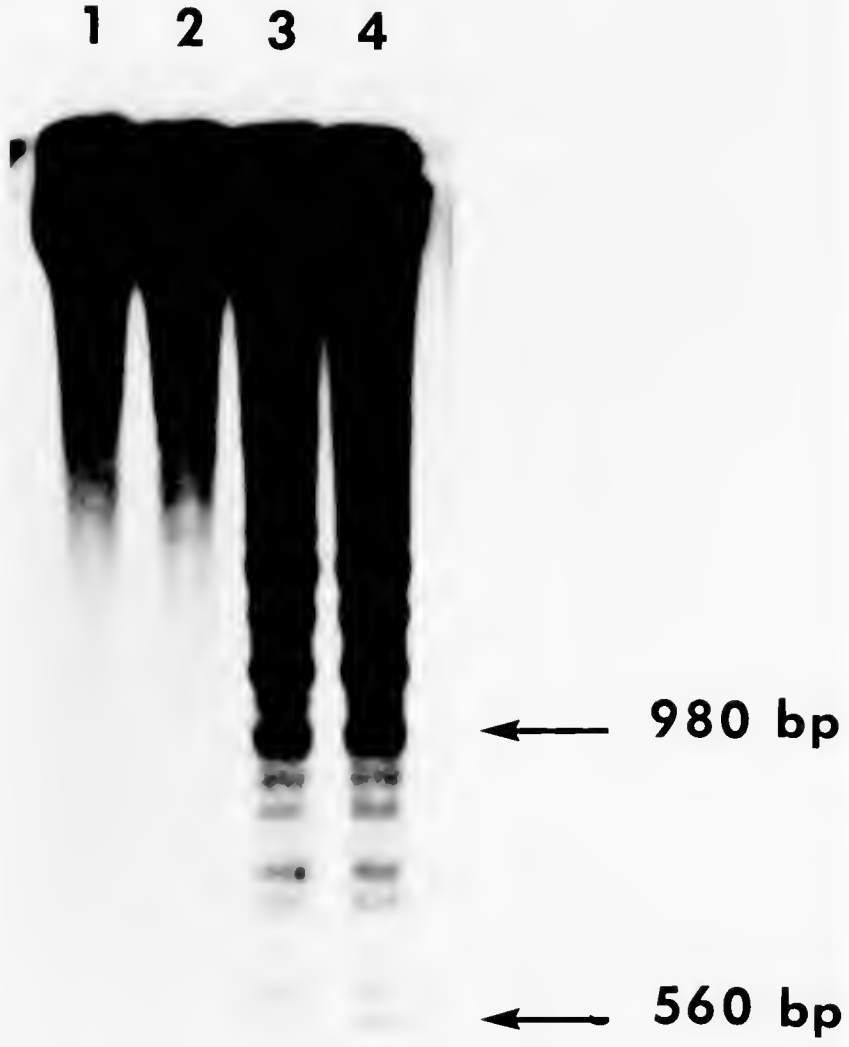

1 2 3 4 5 6 7 8 9 10 11 12
320 bp
280 bp

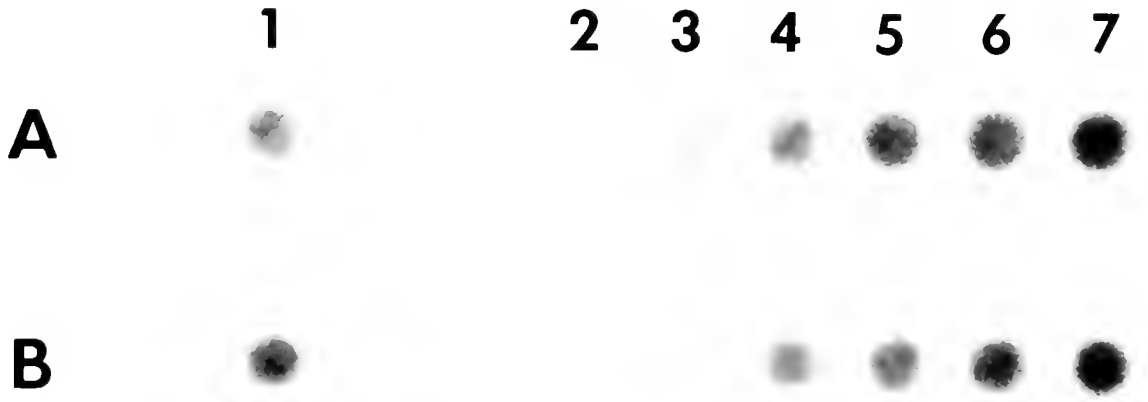
Fig. 8. Hybridization pattern of *P. purpureum* DNA digested with isoschizomers to detect methylation of *Kpn* I families. Total DNA from *P. purpureum* (PI300086) was digested with either *Eco* RII or *Bst* NI, electrophoresed in 2% agarose, Southern blotted, probed at 65° C with nick translated *P. glaucum* IB23 140 bp *Kpn* I monomers, and washed at 65° C in 0.07X SSPE and 0.1% SDS. All lanes contained 2 ug of DNA. Bands of hybridization which migrate at 560 bp and 980 bp are labeled. Lane 1 is *Eco* RII digested DNA. Lane 2 is DNA digested along with the DNA in lane 1, extracted, precipitated, and redigested with *Eco* RII. Lane 3 is *Bst* NI digested DNA. Lane 4 is DNA digested along with the DNA in lane 3, extracted, precipitated, and redigested with *Bst* NI.


1
2
3
4
980 bp
560 bp

I repeat families to the extent that Bst NI did. The restriction endonuclease isoschizomers used here test for methylation at the trinucleotide CNG, N being any nucleotide. This experiment indicates that the Kpn I families of tandemly arrayed repetitive sequences within the P. purpureum nuclear genomes are heavily methylated.

The percentage of the P. purpureum genotype PI300086 (Fig. 9, panel A) and P. glaucum Tift 23 (Fig. 9, panel B) genomes comprised of the Kpn I repeat families was determined along with copy numbers per cell. Various amounts of either P. purpureum 140 bp and 160 bp Kpn I monomers or P. glaucum 140 bp Kpn I monomers were used to create a standard curve. The nuclear DNA of either species was then compared to the standard curve created for each of the species. A dot blot apparatus was used to facilitate the binding of samples to the membrane for probing. The dot blot for the P. purpureum samples was probed with 140 bp and 160 bp Kpn I monomers from P. purpureum. The dot blot for the P. glaucum samples was probed with 140 bp Kpn I monomers from pearl millet. For napiergrass and pearl millet, 100 nanograms of nuclear DNA (lane 1) was compared to various amounts of monomers; the various amounts were 5, 10, 15, 20, 25, and 30 nanograms in lanes 2-7 respectively. For P. purpureum genotype PI300086, 16.4 +/- 1.5 percent of the nuclear genomes are composed of Kpn I repetitive element hybridizing sequences. The P. glaucum genotype Tift 23 has 19.9 +/- 0.8 percent of the nuclear

Fig. 9. Quantitation of the percentage of *P. purpureum* and *P. glaucum* genomes composed of *Kpn* I repeat hybridizing sequences. One hundred ng of nuclear DNA was compared to various amounts of isolated *Kpn* I family monomers by the amount of hybridization to nick translated *Kpn* I family sequences. DNA samples were bound to a nylon filter using a dot blot apparatus. The filters were hybridized at 65^0 C and washed at 65^0 C in 0.07X SSPE and 0.1% SDS. For both A and B, lane 1 is 100 ng of nuclear DNA and lanes 2 through 7 are 5 ng, 10 ng, 15 ng, 20 ng, 25 ng, and 30 ng of *Kpn* I family monomers respectively. Panel A is *P. purpureum* (PI300086) nuclear DNA in lane 1 and a combination of 140 bp and 160 bp *Kpn* I family monomers in the other lanes; the probe DNA was the same combination of *Kpn* I monomers. Panel B is *P. glaucum* Tift 18DB nuclear DNA in lane 1 and *P. glaucum* Tift 23 140 bp *Kpn* I family monomers in the other lanes; the probe DNA was the same preparation of *Kpn* I monomers as those bound to the membrane.


1
2
3
4
5
6
7
A
B

genome composed of Kpn I repetitive element hybridizing sequences. Reassociation kinetics have indicated that about 20% of the pearl millet genome is comprised of the highly repetitive class of sequences (Patankar et al. 1985); the 140 bp Kpn I tandemly arrayed repetitive sequences are thus the major highly repetitive DNA sequences in P. glaucum. The Kpn I families of P. purpureum are likely the major repetitive sequence families within the nuclear genomes as well. Based upon a mole of nucleotide pairs averaging 618 grams, one picomole of DNA equals 9.74×10^8 bp. P. purpureum nuclear DNA is approximately 5.78 picograms (Taylor and Vasil 1987). Thus there are 6.2×10^6 copies of Kpn I repeats per cell. P. glaucum, which contains approximately 4.9 picograms per cell (Patankar et al. 1985), has 6.8×10^6 copies of Kpn I repeats per cell.

To compare the average sequences (Grellet et al. 1986) of the 140 bp and 160 bp Kpn I families of P. purpureum (PI300086), P. glaucum Tift 23, P. squamulatum (PS26), and P. hohenackeri (PS156), either four or five individual Kpn I monomers isolated from individual bands from a 3% polyacrylamide gel were sequenced. The 140 bp Kpn I average sequences from P. squamulatum and P. glaucum are compared to the P. purpureum average sequence in Fig. 10. This is sequence data from one individual plant of each species. Where there is an n in the P. purpureum or P. squamulatum average sequences the bases of two of the four sequences at that position were

Fig. 10. Comparison of the average sequence (Grellet et al. 1986) of P. purpureum (PI300086) to the average sequences of P. squamulatum (PS26) and P. glaucum Tift 23. Average sequences of P. purpureum and P. squamulatum are composed of sequences of four individual Kpn I family monomers. The P. glaucum average sequence is composed of five individual sequences. The three sequences are aligned for comparison purposes. The P. squamulatum and P. glaucum sequences are compared to the P. purpureum average sequence. Differences between the sequences are designated by an asterisk above the position of the base difference in the sequence being compared to the P. purpureum average sequence. In the P. purpureum and P. squamulatum average sequences the appearance of an n is due to their being two bases equally represented at that position; if one of these two bases is the same as the base at the identical position in a sequence being compared then it is not considered a difference between the two sequences.

Comparison Of Average 140 bp Kpn I Sequences

```
1         10        20        30        40        50        60
.         .         .         .         .         .         .
P. purpureum
GGTACCCCGAAATAGTGCATTCAGGnCCGAAACACAAGTTTTGCATCTTTTTACGTGCCG
                          C
                          T
P. squamulatum
GGTACCCCGAAATAGTGCATTCAGGCCCGAAACACAAGTTTTGCATCTTTTTACGTGCCn
                                                            A
                                                            G
P. glaucum                                                  *
GGTACCCCGAAATAGTGCATTCAGGCCCGAAACACAAGTTTTGCATCTTTTTACCTGCCG


          70        80        90        100       110       120
          .         .         .         .         .         .
P. purpureum
AAGGTTAGCGAAATGCTCCGAAACACTCCCAAACATCATTTTGGTTCCAATGGAGTAGAA

P. squamulatum                                      *
AAGGTTAGCnAAATGCTCnGAAACACTCCCAAACATCATTTTGGGTCCAATGGAGTAGAA
         A         C
         G         T
P. glaucum                                          *         *
AAGGTTAGCGAAATGCTCCGAAACACTCCCAAACATCATTTTGGGTCCAATGGACTAGAA


         130
          .
P. purpureum
TGGATGCTTCGCAACTTT

P. squamulatum
          *
TGGATGCTTCACAACTTn
                  C
                  T
P. glaucum
          *       *
TGGATGCTTCACAACTTC
```

one particular base and the bases in the other two sequences at that position were of another particular base. The asterisks in Fig. 10 represent bases in the aligned sequences that differ from the P. purpureum average sequence. If there is an n at one of the positions in the P. purpureum average sequence or the other aligned sequences, and one of the two bases underneath the n is in common, then this is not considered a difference in the comparison.

The individual sequences used in this comparison among these three species varied in length, sequence, and AT content. The average sequences are depicted as 138 bp in length. But these Kpn I families are termed 140 bp families because individual fragments vary in size. The P. purpureum average sequence is 55.4% AT with individual sequences differing from the average by 4.3% to 8.0%. The average sequence for P. squamulatum is 54.3% AT with individual sequences differing from the average by 1.5% to 6.5%. The P. glaucum average sequence is 54.3% AT with individual sequences differing from the average by 8.0% to 10.19%.

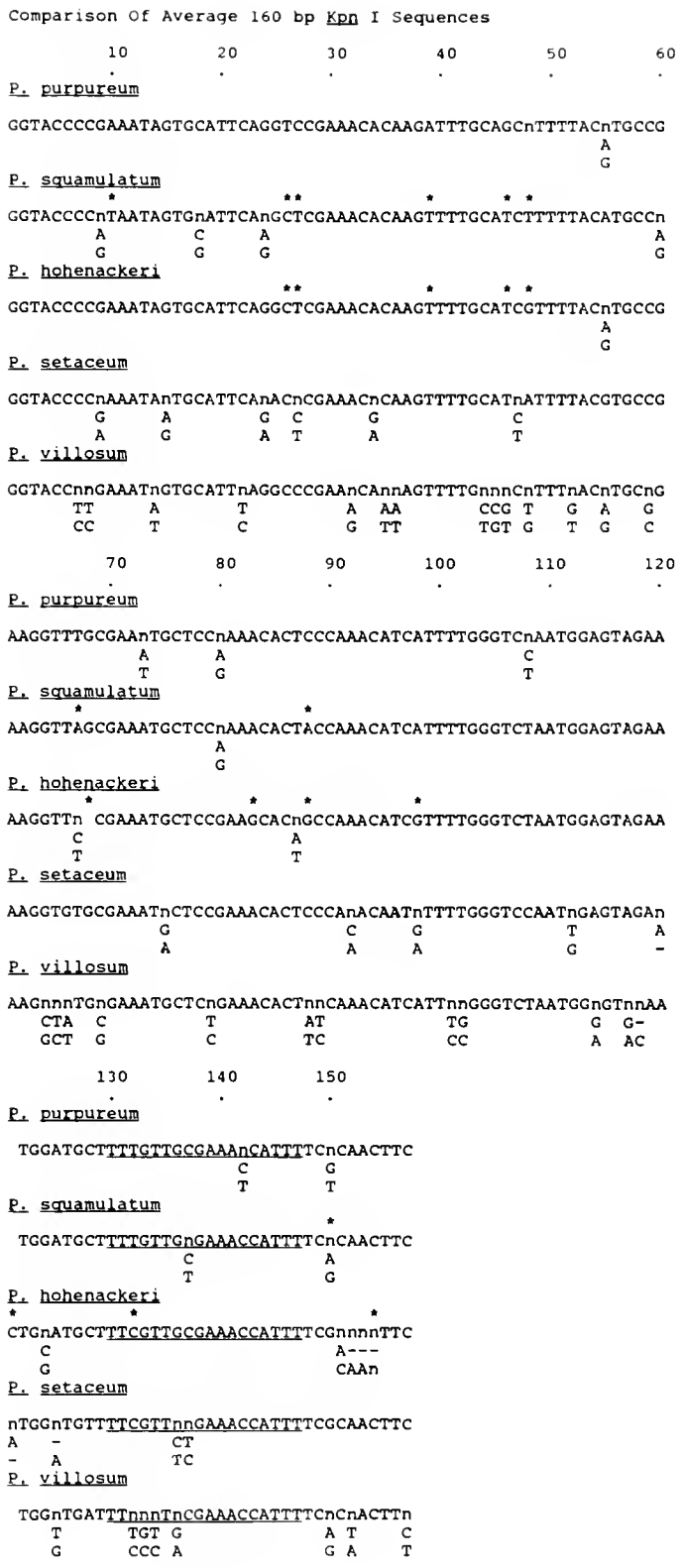
The sequences in Fig. 10 which are compared to the P. purpureum average sequence exhibit close similarity. There are only two positions which distinguish the P. squamulatum 140 bp Kpn I average sequence from that of P. purpureum. The P. glaucum average sequence varies from the P. purpureum average sequence at 4 positions; two of these four differences are the same differences as that of P. squamulatum. The conclusion

from these data is that the P. purpureum, the P. squamulatum, and the P. glaucum 140 bp Kpn I families are closely related to each other.

The average 160 bp Kpn I sequences of P. squamulatum and P. hohenackeri are compared to the average sequence of P. purpureum in Fig. 11. The major difference between the 140 bp and 160 bp Kpn I families is an 18 bp segment located between base numbers 130 and 147. This segment of the sequence is underlined. A couple of individual 160 bp Kpn I fragments from both P. setaceum and P. villosum were sequenced as well to determine how similar these repeat families are to the 160 bp Kpn I families of the above species. The P. setaceum and P. villosum sequences are not average sequences; hence they are not compared to the P. purpureum average sequence. Differences between the two individual sequences for these two species are denoted by an n; the actual bases observed are listed below the n. The format up of Fig. 11 is similar to that of Fig. 10. The variable position in the P. purpureum average sequence at base number 48 was different in all four individual fragments sequenced.

As in the case of the 140 bp Kpn I fragments, the 160 bp Kpn I individual fragments varied from each other in length, sequence, and AT content. The AT content of the P. purpureum average sequence is 56.1% while the individual sequences varied from the average by 4.5% to 14.1%. The P. squamulatum average sequence is 55.8% AT while the individual sequences

Fig. 11. Comparison of the average sequence (Grellet et al. 1986) of P. purpureum (PI300086) to the average sequences of P. squamulatum (PS26) and P. hohenackeri (PS156). All average sequences are composed of four individual Kpn I family monomers. Also shown in this figure are sequences from two individual Kpn I family monomers from P. setaceum (PS247) and P. villosum (PS249); differences between these two sequences are represented as an n with the two bases below the n being from the two sequences. The sequences are aligned so that the insertion and the deletion present in the P. hohenackeri average sequence can be compared to the other average sequences. Differences between the average sequences are designated by an asterisk above the position of the base difference in the average sequence being compared to the P. purpureum average sequence. In the average sequences the appearance of an n is due to their being two bases equally represented at that position; if one of these two bases is the same as the base at the identical position in a sequence being compared then it is not considered a difference between the two sequences. All four individual sequences were different at position 48 in the P. purpureum average sequence. Bases from position 130 to 148 are underlined in all six sequences. This 18 bp underlined region represents the major difference between the 140 bp and 160 bp Kpn I family sequences.


Comparison Of Average 160 bp Kpn I Sequences
P. purpureum
P. squamulatum
P. hohenackeri
P. setaceum
P. villosum

varied from the average by 3.8% to 13.5%. The AT content for the P. hohenackeri average sequence is 52.9% while the individual sequences varied from the average by 1.3% to 13.5%. The AT contents for the two P. setaceum individual sequences are 56.1% and 59.4%; these two sequences differ from each other by 9.6%. The AT content for the two P. villosum individual sequences are 58.3% and 53.8% while the sequences differ by 21.8%.

Comparison of the average sequences of P. squamulatum and P. hohenackeri to P. purpureum indicates that the 160 bp Kpn I family of P. squamulatum is the more closely related. As seen in the aligned average sequence comparison, P. purpureum differed from P. squamulatum by nine bases. These are indicated by asterisk. The P. hohenackeri average sequence differs from the P. purpureum average sequence at 15 positions including a deletion at base 68 and an insertion at base 121. P. hohenackeri had two individual sequences with an A at position 151 without any bases corresponding to positions 152-154 as compared to the P. purpureum average sequence; the other two individual sequences had CAAC and CAAG at positions 151-154. All four P. hohenackeri individual fragments sequenced were included in the average sequence. These sequence data suggest that the P. purpureum (PI300086) and P. squamulatum (PS26) Kpn I families diverged after the divergence from the P. hohenackeri (PS156) Kpn I family.

Grellet et al. (1986) have constructed an average sequence of a radish (Raphanus sativus) tandemly arrayed repetitive sequence family of 177 bp. This sequence was shown to have three subrepeats of about 60 bp; it was also shown to have limited similarity to other plant tandem repeats and animal alphoid sequences. Subrepeats within the P. purpureum average 160 bp Kpn I sequence were observed (Fig. 12). These subrepeats are aligned according to conserved regions within the individual subrepeats. The base numbers of the subrepeats are relative to the average 160 bp Kpn I sequence of P. purpureum in Fig. 11. There are five conserved regions of three bases or more indicated by the solid lines underneath the individual subrepeats. As can be observed, these subrepeats may have undergone rearrangements involving insertions, deletions, and substitutions. Similarity between the P. purpureum 160 bp Kpn I subrepeats and a maize satellite DNA monomer (Peacock et al. 1981) is depicted in Fig. 12 as well. Four of the five conserved regions in the P. purpureum subrepeats are also present in the segment of the maize monomer below the subrepeats. Those regions conserved between the P. purpureum and maize sequences are underlined below the maize sequence. When maize DNA was digested with Kpn I, electrophoresed, Southern blotted, and probed with pearl millet 140 bp Kpn I sequences no hybridization was detected. Also probed with pearl millet 140 bp Kpn I monomers was sorghum and several members of the Saccharum complex; again,

Fig. 12. Subrepeats within the P. purpureum average 160 bp Kpn I sequence were aligned according to conserved regions present in these subrepeats. These conserved regions present in the subrepeats are underlined below each subrepeat. Below the P. purpureum subrepeats is a maize satellite DNA monomer segment (Peacock et al. 1981). The regions of similarity between the P. purpureum subrepeats and the maize satellite DNA monomer segment are underlined below the maize sequence. The maize sequence is aligned such that the positioning of the conserved regions between the species can be compared to the conserved regions from the subrepeats.

Aligned Subrepeats Within the Pennisetum purpureum Average 160 bp Kpn I Sequence; Similarity to a Maize Repeat is Also Shown

Bases 85-142

```
ACTCCCAAA CATCATTTTGGGT Cn AATGGAGTA  GAATGGATGCTTTT GTTGCGAAAn
                        C                                     C
                        T                                     T
```

Bases 143-33

```
             CATTTTCnCAACTTC  GGTACCCCGAAATAGTGCATTCAGGTCCGAAAC
                    G
                    T
```

Bases 34-84

```
ACAAGATTTGCAGCnTTTTACnTGCCGAA GGTTTGC GAAn   TGC       TCCnAAAC
                      A                 A                 A
                      G                 T                 G
```

Maize Satellite DNA Monomer Segment Bases 68-139

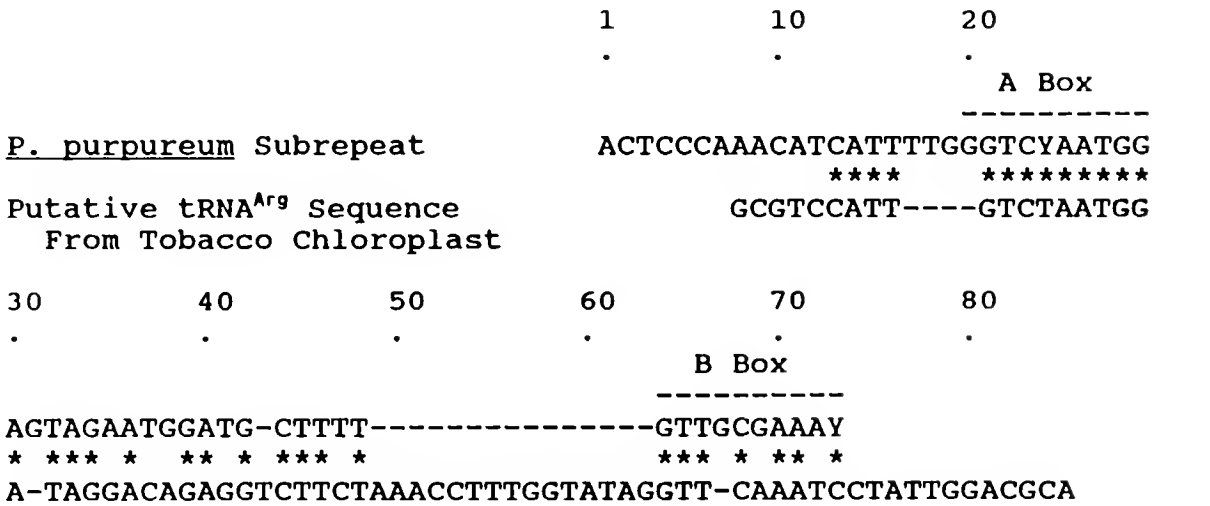
```
   ACACCTACGGATTTTTGACCAAGAAATGGTCTCCACCAGAAAATCCAA
                                        GAATGTGATCTAGGCAAGGAAAC
```

no hybridization was detected indicating a lack of Kpn I hybridizing sequences within the genomes of these species (data not shown). The P. purpureum average 160 bp Kpn I sequence is composed of subrepeats; the conserved regions within these subrepeats are also present in a maize satellite DNA monomer. The P. purpureum and maize repeats in Fig. 12 may have had a common origin.

The origin of plant tandemly arrayed repetitive sequences are transfer RNAs according to Benslimane et al. (1986). The repeats may have arisen from a tRNA progenitor by reverse transcription followed by rolling circle replication. When a GenBank search was conducted with the entire P. purpureum average 160 bp Kpn I sequence the strongest similarity was found with a putative tobacco chloroplast $tRNA^{Arg}$ gene (Deno and Sugiura 1984). To determine if the subrepeats of the P. purpureum average 160 bp Kpn I sequence have similarity to tRNAs or any other sequence a GenBank search was performed with bases 85 to 142 from the P. purpureum average 160 bp Kpn I sequence (the top subrepeat in Fig. 12). This GenBank search detected sequences which are similar to only part of the 160 bp Kpn I average sequence represented as a subrepeat verses the entire average sequence. The result of the GenBank search with the subrepeat differed from the GenBank search with the entire 160 bp Kpn I average sequence. The strongest similarity was found to a maize mitochondrial 5 kb repeat (Houchins et al. 1986) with no close similarity to any tRNAs. For both

Fig. 13. The P. purpureum average 160 bp Kpn I sequence subrepeat from bases 85 to 142 is compared to a putative tobacco chloroplast $tRNA^{Arg}$ sequence (Deno and Sugiura 1984). The subrepeat is 57 bp and the tRNA is 72 bp in length. Within the sequences the gaps are necessary to align the sequences for comparison; these gaps are indicated as dashes (-) within the sequences. Asterisks indicate matching bases between the two sequences. Over the subrepeat sequence the regions of the compared sequences with homology to the two RNA polymerase III internal promoter elements, A box and B box, are indicated. Below the comparison of the subrepeat to the tRNA is a comparison of the subrepeat A box and B box like regions to the RNA polymerase III internal promoter consensus sequences (Joyce et al. 1988). In the internal promoter A box consensus sequence the two dashes (-) indicate that there may or may not be bases present in those positions. Asterisks indicate matching bases between the subrepeat regions and the internal promoter consensus sequences. A Y indicates that the base at that position is a pyrimidine; a R indicates that the base at that position is a purine.

Comparison of a Pennisetum purpureum Subrepeat to a tRNA Sequence and to RNA Polymerase III Internal Promoter Elements



	A Box	B Box
P. purpureum Subrepeat	GGTCYAAT--GG	GTTGCGAAA$^{T}_{C}$
RNA Polymerase III Internal Promoter Consensus Sequences	RGYNNRRY--GG	G$^{T}_{A}$T CRANNC

GenBank searches FASTA was used (Pearson and Lipman 1988) with a K-tuple size of three.

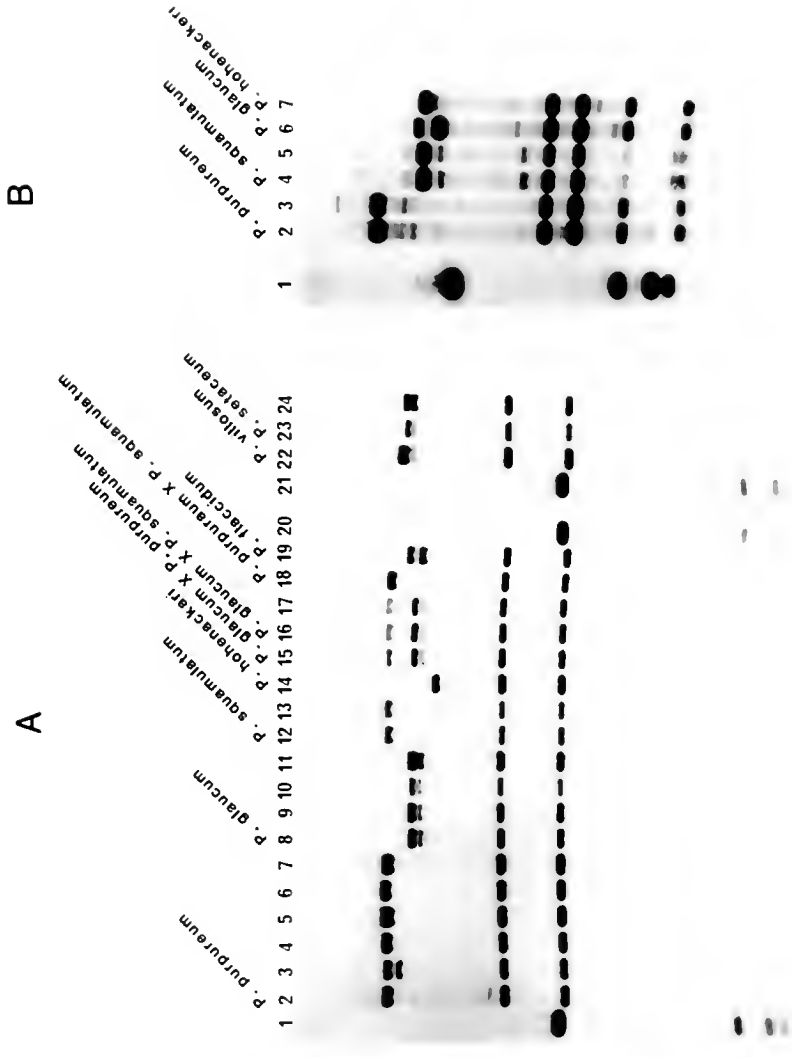
A P. purpureum subrepeat (from base 85 to 142 of the average sequence) is compared to the $tRNA^{Arg}$ sequence mentioned above in Fig. 13. The asterisk between the two sequences indicate bases in common. Thirty two of the 57 bases comprising the P. purpureum subrepeat in Fig. 13 agree with the bases in the tobacco tRNA coding region; this amounts to a 56.1% conservation of bases. The subrepeat including bases 85 to 142 of the P. purpureum average sequence when aligned to the tRNA has two regions of the possible tRNA progenitor missing. The first missing region is the T loop side of the anticodon loop (bases 49-63) seen as a gap represented with dashed lines in the subrepeat sequence; the second is the T loop side of the aminoacyl stem which would extend past the B box region of the subrepeat sequence.

Part of the conserved regions include sequences having homology to the RNA polymerase III internal promotor elements: A box and B box. The region of the P. purpureum subrepeat having homology to the A box has a dashed line above it; this region is also compared to the RNA polymerase III A box consensus sequence (Joyce et al. 1988) below the sequence comparison. The A box in the P. purpureum subrepeat has homology to 7 out of 7 conserved bases within the RNA polymerase III A box consensus sequence. The B box in the P. purpureum subrepeat (also indicated by a dashed line above the

subrepeat sequence) has homology to 8 out of 8 conserved bases within the RNA polymerase III B box consensus sequence with an insertion of one base, this is shown below the sequence comparison. It appears that the subrepeats composing the Kpn I families of P. purpureum and the other Pennisetum species characterized arose from a tRNA progenitor; the presence of the transcriptional control elements within these subrepeats is fairly conclusive. The maize satellite DNA segment in Fig. 12 also has regions similar to the A box and B box, but with limited sequence identity.

Ribosomal DNA RFLPs are depicted in Fig. 14. Total DNA from seven Pennisetum species and four interspecific hybrids (Fig. 14A) were digested with Sst I, electrophoresed, blotted, and probed with a maize rDNA repeat containing the coding regions for the 17s, 5.8s, and 26s rRNA genes (McMullen et al. 1986). The variable bands are interpreted as differences in the size of the intergenic spacer (IGS) region of the rDNA repeats. The data are compatible with the idea that the size of the variable band is due to the number of subrepeats within the IGS. This figure (14A) displays the rDNA RFLPs within and between Pennisetum species with an emphasis on the similarities between P. purpureum and P. squamulatum hybridization patterns. Lanes 2-7 are six genotypes of P. purpureum; rDNA repeat length heterogeneity is seen only in genotype N16. All of the P. purpureum genotypes have a rDNA repeat size of 9.1 kb when all three major bands are totaled;

Fig. 14. Ribosomal DNA RFLPs in Pennisetum species and interspecific hybrids. Total DNAs were probed with a maize rDNA repeat to examine rDNA RFLPs. (A) DNAs (1 ug) from Pennisetum species and interspecific hybrids were digested with Kpn I, electrophoresed in 0.8% agarose, Southern blotted, probed at 60^0 C with random primer labeled maize rDNA repeat coding region (McMullen et al. 1986), and washed at 60^0 C in 0.1X SSPE and 0.1% SDS. Lanes 1, 20, and 21 are 1 kb markers. Total DNAs are P. purpureum (PI300086) (lane 2), P. purpureum (N16) (lane 3), P. purpureum (N137) (lane 4), P. purpureum (N138) (lane 5), P. purpureum (N166) (lane 6), P. purpureum Merkeron (lane 7), P. glaucum Tift 23 (lane 8), P. glaucum Tift 23A (lane 9), P. glaucum ssp. monodii (PS34) (lane 10), P. glaucum ssp. monodii (PS470) (lane 11), P. squamulatum (PS24) (lane 12), P. squamulatum (PS26) (lane 13) P. hohenackeri (PS156) (lane 14), P. glaucum Tift 23A X P. purpureum (N16) (lane 15), P. glaucum Tift 239DB X P. squamulatum (PI319196) (lane 16), P. glaucum (H165) X P. squamulatum (PS26) (lane 17), P. purpureum Merkeron X P. squamulatum (PS262) (lane 18), P. flaccidum (PS396) (lane 19), P. villosum (PS249) (lane 22), P. setaceum (PS49) (lane 23), and P. setaceum (PS247) (lane 24). (B) DNAs (1 ug) from Pennisetum species were digested with Bst N1, electrophoresed in 1.25% agarose, Southern blotted, probed at 62^0 C with random primer labeled maize rDNA repeat coding region, and washed at 62^0 C in 0.1X SSPE and 0.1% SDS. Lane 1 is 1 kb markers. Total DNAs are P. purpureum (PI300086) (lane 2), P. purpureum (N16) (lane 3), P. squamulatum (PS24) (lane 4), P. squamulatum (PS26) (lane 5), P. glaucum Tift 23 (lane 6), and P. hohenackeri (PS156) (lane 7).


A
B
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
P. purpureum
P. glaucum
P. squamulatum
P. hohenackeri
P. glaucum x P. purpureum
P. glaucum x P. squamulatum
P. purpureum x P. squamulatum
P. flaccidum
P. villosum
P. setaceum
1 2 3 4 5 6 7
P. purpureum
P. squamulatum
P. glaucum
P. hohenackeri

genotype N16 also has a repeat of 8.6 kb. P. purpureum, an allotetraploid, has two bivalents associated with the nucleolus during diakinesis of meiosis (Jauhar 1981). The P. purpureum hybridization pattern indicates there has been homogenization of rDNA repeat length between nonhomologous chromosomes. In lanes 8 and 9 are two related pearl millet genotypes; lanes 10 and 11 are two genotypes of P. glaucum ssp. monodii. The hybridization patterns between the cultivated and wild subspecies of P. glaucum are the same although the hybridization intensities differ. There are two major repeat sizes in the P. glaucum genotypes. These are 8.0 kb and 7.8 kb. This diploid species, which contains one bivalent associated with the nucleolus during diakinesis of meiosis (Jauhar 1981), exhibits greater repeat length heterogeneity than the allotetraploid P. purpureum. This rDNA repeat length heterogeneity exists on homologous chromosomes. Lanes 12 and 13 are two genotypes of the apomictic hexaploid P. squamulatum. As can be seen, there is one major band of hybridization representing the IGS region, the major repeat has a length of 9.1 kb. This hybridization pattern is identical to P. purpureum except that P. squamulatum has two minor bands of 4.0 kb and 2.8 kb. There are three bivalents associated with the nucleolus during diakinesis of meiosis in P. squamulatum (Jauhar 1981). Again, as in P. purpureum, there appears to be a homogenization of rDNA repeat length between nonhomologous chromosomes. One P. hohenackeri genotype was

characterized (lane 14). P. hohenackeri contains an Sst I site not present in the rDNA repeats of other Pennisetum species observed. The P. hohenackeri rDNA repeat may have diverged prior to divergence of the other Pennisetum species shown here. When the four major bands of hybridization are totaled, P. hohenackeri appears to have a rDNA repeat length of 8.2 kb. The interspecific hybrid P. glaucum Tift 23A X P. purpureum (N16) hybridization pattern is in lane 15. Only one of the two repeat sizes in the P. purpureum parent N16 is present in the triploid. This indicates the P. purpureum (N16) repeat length heterogeneity seen in lane 3 exists on homologous chromosomes and not on nonhomologous chromosomes because homologous chromosomes segregate during meiosis. Other than the lack of the 8.6 kb repeat from the P. purpureum parent, the hybridization pattern is additive between the parents. Lane 16 is the interspecific hybrid P. glaucum Tift 239DB X P. squamulatum (PI319196) while lane 17 is P. glaucum (H165) X P. squamulatum (PS26). Only the P. squamulatum (PS26) parent is shown in this figure; however, the hybridization pattern of the above P. glaucum X P. squamulatum hybrid is the expectation of the combination of any of the P. glaucum or P. squamulatum genotypes present in this figure. A P. purpureum Merkeron X P. squamulatum (PS262) hybrid is shown in lane 18. This hybridization pattern displays comigration of the major repeat sizes of these two species indicating very similar if not identical sized rDNA repeats. The next four lanes of

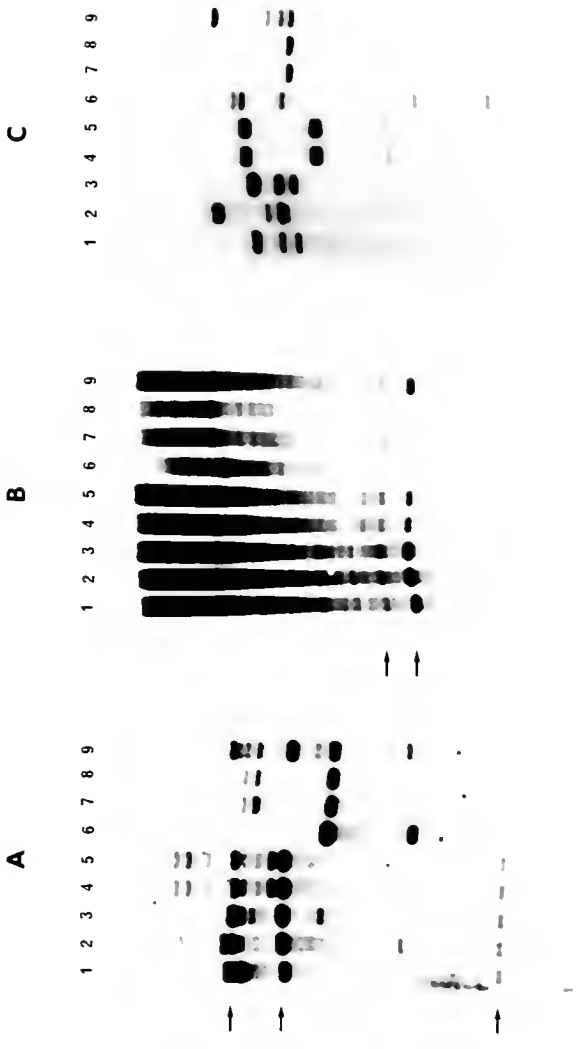
Pennisetum species (lanes 19, 22, 23,and 24) are P. flaccidum, P. villosum, and two genotypes of P. setaceum respectively. The major repeat sizes of P. flaccidum are 8.2 kb and 7.9 kb. The Two major repeats are 8.5 kb and 8.1 kb for the P. villosum (lane 22). The first P. setaceum has major repeats of 8.3 kb and 8.1 kb. With the second P. setaceum (lane 24) the major rDNA repeats are 8.4 kb and 8.1 kb. There exists little intraspecific heterogeneity of rDNA repeat length or RFLPs within these species except for P. purpureum (N16) and the two P. setaceum species. However, there is intragenic heterogeneity of rDNA repeat length between all these Pennisetum species with the exception of P. purpureum and P. squamulatum. The Sst I RFLPs between P. purpureum and P. squamulatum may indicate a close relationship between these rDNA repeat families. In maize and maize relatives rDNA repeat length variation is not phylogenetically informative (Zimmer et al. 1988). However, in Lisianthius species rDNA length variation can be used as an indication of relatedness (Systma and Schaal 1985).

P. purpureum and P. squamulatum rDNA repeats were further compared. Total DNAs were digested with Bst NI, electrophoresed, blotted, and probed with the same maize repeat used to probe the Sst I digest (Fig. 14B). Lanes 2 and 3 are two P. purpureum genotypes; lanes 4 and 5 are two P. squamulatum genotypes; lane 6 is P. glaucum; lane 7 is P. hohenackeri. The RFLPs differ among all four of these

Pennisetum species. While P. purpureum and P. squamulatum have similar Sst I rDNA RFLPs, Bst NI digestion indicates sequence divergence has occurred. The rDNA RFLPs of Bst NI digested Pennisetum DNAs do not indicate as close a similarity between P. purpureum and P. squamulatum rDNA repeats as the Sst I hybridization pattern indicates.

To further compare the nuclear genomes of P. purpureum, P. squamulatum, P. glaucum, and P. hohenackeri RFLPs were observed when the above Pennisetum DNAs were probed with cloned sequences from napiergrass. For Fig. 15A, B, and C, DNAs were digested with Eco RI, electrophoresed, blotted, and probed. All three panels in Fig. 15 have the same order of samples. The first three lanes are different genotypes of P. purpureum; lanes 4 and 5 are two genotypes of P. squamulatum; lane 6 is P. hohenackeri, lanes 7 and 8 are two P. glaucum genotypes; and lane 9 is a P. glaucum X P. purpureum triploid hybrid. The DNAs in Fig. 15A were probed with a genomic sucrose synthase clone from P. purpureum. The RFLP similarities between P. purpureum and P. squamulatum are evident. These similarities between the hybridization patterns are marked by the three arrows. Only the P. squamulatum RFLPs are similar to P. purpureum. P. glaucum and P. hohenackeri exhibit no common RFLPs when compared to P. purpureum. In Fig. 15B a close relationship between the genomes of P. purpureum and P. squamulatum is evident. The probe for this panel was a random cDNA clone from P. purpureum leaf tissue. This clone

Fig. 15. RFLPs in Pennisetum species and an interspecific hybrid. Total DNAs (2 ug) from Pennisetum species were digested with Eco R1, electrophoresed in 0.8% agarose, Southern blotted, probed at 68^0 C with random primer labeled clones from P. purpureum, and washed at 68^0 C in 0.1X SSPE and 0.1% SDS. Only the probes differed in the three experiments shown in A, B, and C. Arrows indicate common zones of hybridization between the lanes. Total DNAs (for A, B, and C) are P. purpureum (PI300086)(lane 1), P. purpureum (N16) (lane 2), P. urpureum (N137) (lane 3), P. squamulatum (PS24) (lane 4), P. squamulatum (PS26) (lane 5), P. ohenackeri (PS156) (lane 6), P. glaucum Tift 23 (lane 7), P. glaucum Tift 23A (lane 8), and P. glaucum Tift 23A X P. purpureum N16 (lane 9). (A) The probe was a sucrose synthase genomic clone. (B) The probe was a random cDNA clone from leaf tissue. (C) The probe was a random genomic clone.

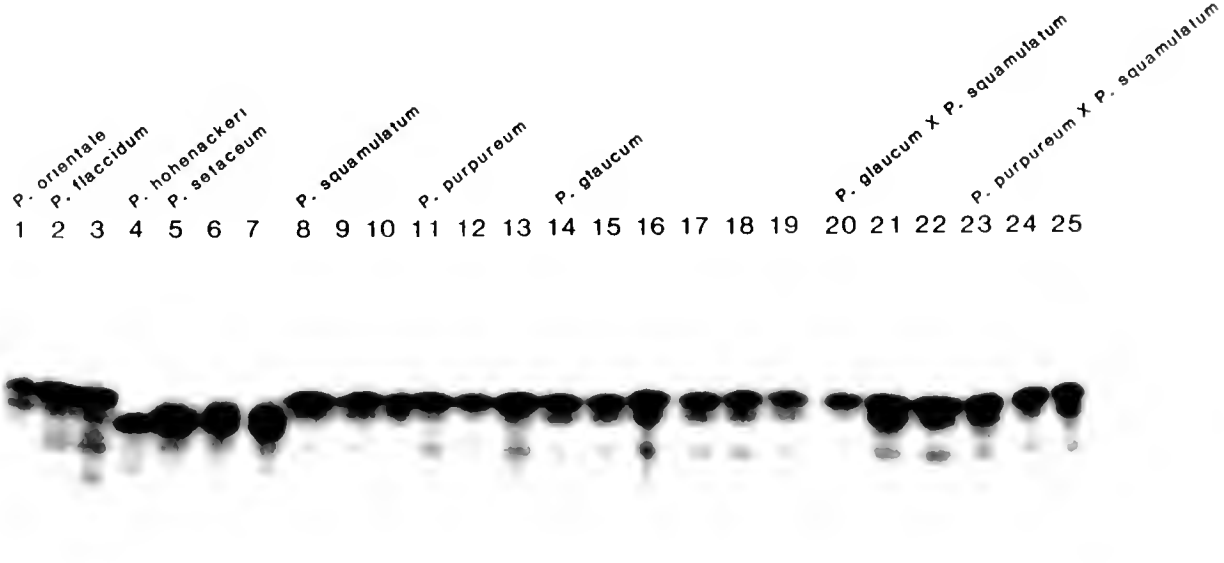

C
1 2 3 4 5 6 7 8 9
B
1 2 3 4 5 6 7 8 9
A
1 2 3 4 5 6 7 8 9

contains a sequence which is repeated many times within the nuclear genome. The top arrow in Fig. 15B designates a band of hybridization seen in the P. purpureum, P. squamulatum, and P. glaucum genotypes. The bottom arrow in Fig. 15B is adjacent to a common band of hybridization in the P. purpureum and P. squamulatum genotypes. A random genomic clone was used to probe the DNAs in Fig. 15C; no common RFLPs were detected in any of the Pennisetum species. Fig. 15 displays additional evidence indicating similarity between the nuclear genomes of P. purpureum and P. squamulatum; at the same time though, there are differences as well. As with the rDNA repeat comparisons, P. hohenackeri shows no relatedness to P. purpureum or P. squamulatum.

Isozyme phenotypes (Figs. 16-20) were observed for various Pennisetum species and interspecific hybrids to identify common zones of activity. In all five of the isozyme gels shown, MDH (malate dehydrogenase), PGI (phosphoglucoisomerase), APS (alkaline phosphatase), GOT (glutamate oxaloacetate transaminase), and ADH (alcohol dehydrogenase) the samples are in the same order except that in the ADH figure there are seven P. glaucum samples instead of six. The direction of electrophoresis is from the bottom to the top. The MDH banding pattern is shown in Fig. 16. Lane 4 is P. hohenackeri while lanes 5-7 are various genotypes of P. setaceum, these plants are closely related phenotypically. These two species exhibit a differential MDH banding pattern

Fig. 16. MDH isozyme banding pattern of Pennisetum species and interspecific hybrids. Protein electrophoresis is from the bottom of the figure towards the top. Leaf extracts were electrophoresed in a starch gel. After electrophoresis the proteins were stained for activity. The order of the samples is P. orientale (PS14) (lane 1), P. flaccidum (PS3) (lane 2), P. flaccidum (PS4) (lane 3), P. hohenackeri (PS156) (lane 4), P. setaceum (PS49) (lane 5), P. setaceum (PS31) (lane 6), P. setaceum (PS22) (lane 7), P. squamulatum (PS24) (lane 8), P. squamulatum (PS26) (lane 9), P. squamulatum (PS158) (lane 10), P. purpureum (PI300086) (lane 11), P. purpureum Merkeron (lane 12), P. purpureum (N14) (lane 13), P. glaucum Tift 23 (lane 14), P. glaucum Tift 18DB (lane 15), P. glaucum Tift 383 (lane 16), P. glaucum Tift 23 (lanes 17, 18, and 19), P. glaucum (176) X P. squamulatum (PS26) (lane 20), P. glaucum (H165) X P. squamulatum (PS26) (lanes 21 and 22), and P. purpureum Merkeron X P. squamulatum (PS262) (lanes 23, 24, and 25).

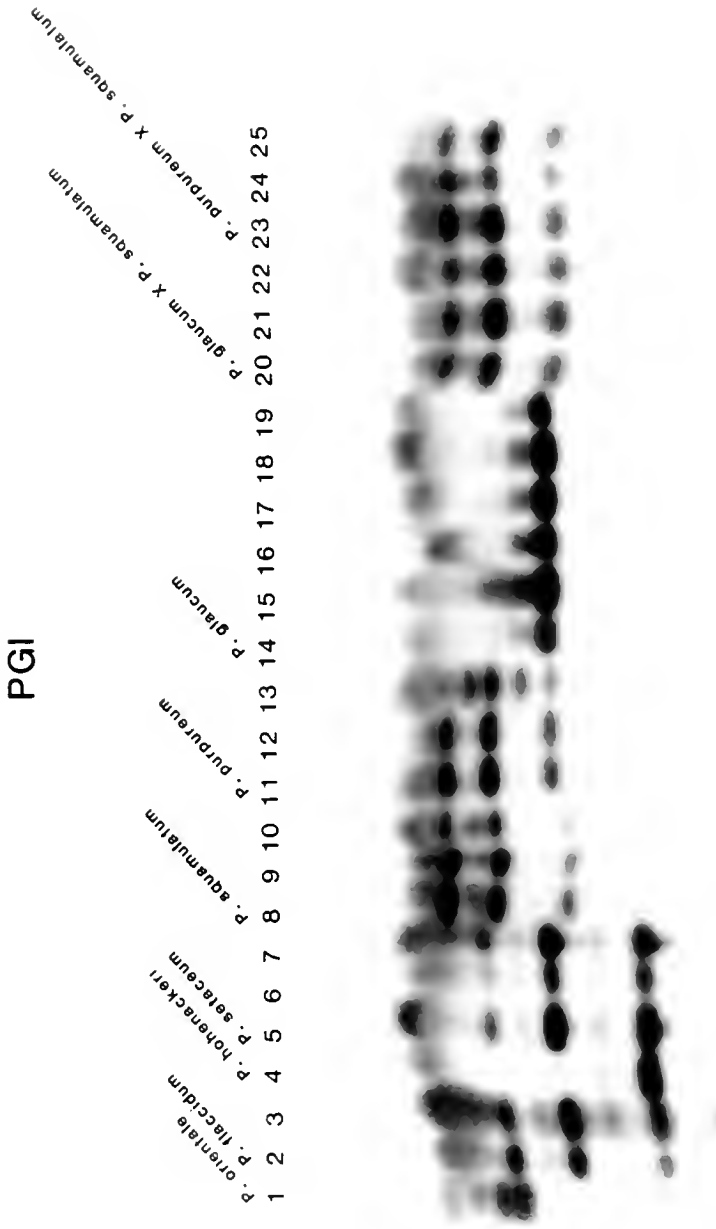
MDH


P. orientale
P. flaccidum
P. hohenackeri
P. setaceum
P. squamulatum
P. purpureum
P. glaucum
P. glaucum X P. squamulatum
P. purpureum X P. squamulatum
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

as compared to the other Pennisetum species. All Pennisetum species and hybrids exhibit four zones of activity. It should be mentioned that three MDH loci have been reported in pearl millet (Tostain et al. 1987). The difference may be related to the buffer systems. Or, it is possible the top bands are from one locus with one band being a ghost band. The ghost band, which migrates differentially, could be due to an alteration in protein structure.

The PGI banding patterns of these Pennisetum species are shown in Fig. 17. P. flaccidum (lanes 2 and 3), P. hohenackeri (lane 4), and P. setaceum (lanes 5-7) all contain the slowest migrating PGI zones of activity among the various species. The above three Pennisetum species, along with P. orientale in lane 1, have distinctly different PGI phenotypes as compared to the three related Pennisetum species: P. squamulatum, P. purpureum, and P. glaucum. The slowest migrating PGI zone of activity present in the P. purpureum genotypes (lanes 11-13) comigrates with a zone of activity present in the pearl millet genotypes. The P. squamulatum PGI phenotypes (lanes 8-10) are similar to the P. purpureum phenotypes (lanes 12-14) except for the P. purpureum zones of activity which comigrate with the P. glaucum zones of activity. P. purpureum N14 in lane 13 appears to have three alleles of the PGI gene as seen by the appearance of more than three zones of activity. All of the P. glaucum PGI phenotypes (lanes 14-19) are similar, the two bands may be from one locus with the top band being a ghost

Fig. 17. PGI isozyme banding pattern of Pennisetum species and interspecific hybrids. Protein electrophoresis is from the bottom of the figure towards the top. Leaf extracts were electrophoresed in a starch gel. After electrophoresis the proteins were stained for activity. The order of the samples is P. orientale (PS14) (lane 1), P. flaccidum (PS3) (lane 2), P. flaccidum (PS4) (lane 3), P. hohenackeri (PS156) (lane 4), P. setaceum (PS49) (lane 5), P. setaceum (PS31) (lane 6), P. setaceum (PS22) (lane 7), P. squamulatum (PS24) (lane 8), P. squamulatum (PS26) (lane 9), P. squamulatum (PS158) (lane 10), P. purpureum (PI300086) (lane 11), P. purpureum Merkeron (lane 12), P. purpureum (N14) (lane 13), P. glaucum Tift 23 (lane 14), P. glaucum Tift 18DB (lane 15), P. glaucum Tift 383 (lane 16), P. glaucum Tift 23 (lanes 17, 18, and 19), P. glaucum (176) X P. squamulatum (PS26) (lane 20), P. glaucum (H165) X P. squamulatum (PS26) (lanes 21 and 22), and P. purpureum Merkeron X P. squamulatum (PS262) (lanes 23, 24, and 25).

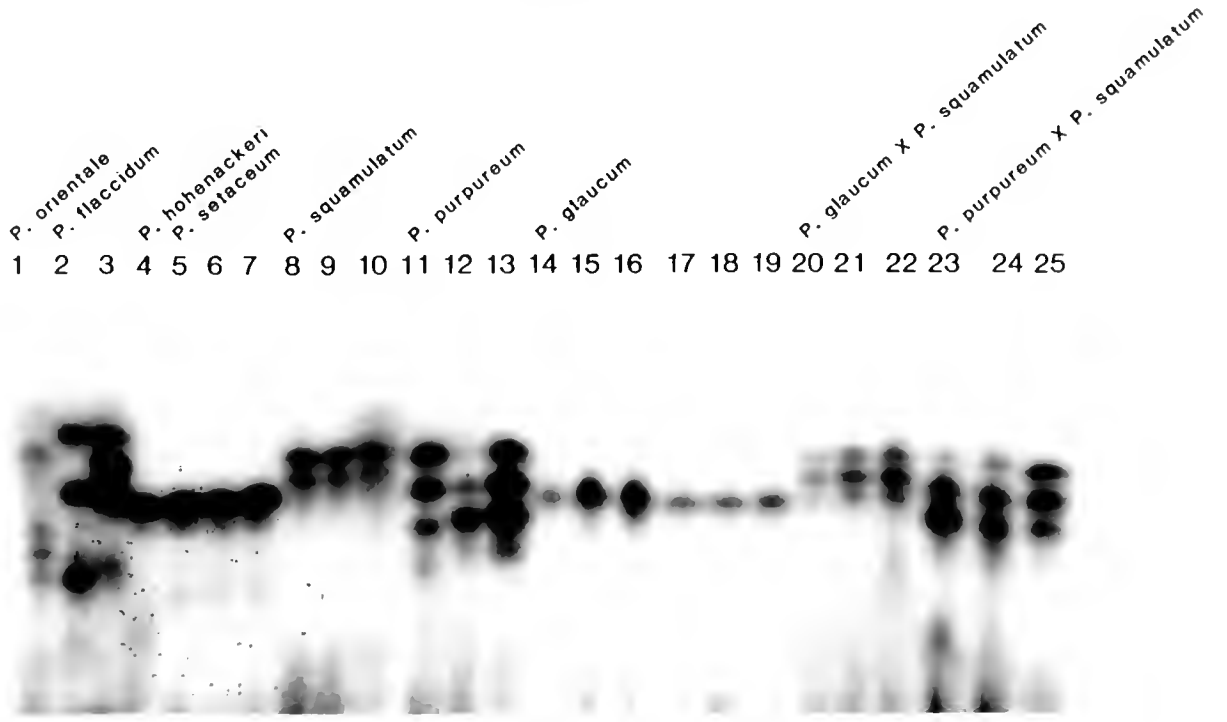

PGI
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
P. ornatus
P. fiaccidum
P. hohenackeri
P. balbisianum
P. squamulatum
P. purpureum
P. glaucum
P. glaucum x P. squamulatum
P. purpureum x P. squamulatum

band. One PGI locus has been identified in pearl millet (Tostain et al. 1987). The P. glaucum X P. squamulatum interspecific hybrids (lanes 20-22) and the P. purpureum X P. squamulatum hybrids (lanes 23-25) have the same PGI banding patterns. The similarity in both sets of interspecific hybrids suggests that P. squamulatum has a gene(s) involved in the posttranslational modification of PGI. This is because the slowest migrating zones of activity in P. purpureum and P. glaucum are more likely altered in mobility rather than not expressed in the P. squamulatum interspecific hybrids. This would explain the difference between the slowest migrating zone of activity present in the P. squamulatum genotypes and hybrids as compared to P. purpureum. The PGI banding patterns show a close relatedness between P. purpureum and P. squamulatum.

The APS banding patterns are shown in Fig. 18. P. purpureum (lanes 11-13) and P. squamulatum (lanes 8-10) have only one comigrating zone of activity; the fastest migrating zone of activity. The two bands in the P. squamulatum phenotypes may be from one locus with one band being a ghost band. It does not appear that the zone of activity present in the P. glaucum genotypes (lanes 14-19) is present in the P. purpureum or P. squamulatum phenotypes. All three of the closely related Pennisetum species, P. glaucum, P. purpureum, and P. squamulatum, have different APS isozyme banding patterns. The P. glaucum X P. squamulatum interspecific

Fig. 18. APS isozyme banding pattern of Pennisetum species and interspecific hybrids. Protein electrophoresis is from the bottom of the figure towards the top. Leaf extracts were electrophoresed in a starch gel. After electrophoresis the proteins were stained for activity. The order of the samples is P. orientale (PS14) (lane 1), P. flaccidum (PS3) (lane 2), P. flaccidum (PS4) (lane 3), P. hohenackeri (PS156) (lane 4), P. setaceum (PS49) (lane 5), P. setaceum (PS31) (lane 6), P. setaceum (PS22) (lane 7), P. squamulatum (PS24) (lane 8), P. squamulatum (PS26) (lane 9), P. squamulatum (PS158) (lane 10), P. purpureum (PI300086) (lane 11), P. purpureum Merkeron (lane 12), P. purpureum (N14) (lane 13), P. glaucum Tift 23 (lane 14), P. glaucum Tift 18DB (lane 15), P. glaucum Tift 383 (lane 16), P. glaucum Tift 23 (lanes 17, 18, and 19), P. glaucum (176) X P. squamulatum (PS26) (lane 20), P. glaucum (H165) X P. squamulatum (PS26) (lanes 21 and 22), and P. purpureum Merkeron X P. squamulatum (PS262) (lanes 23, 24, and 25).

APS


P. orientale
P. flaccidum
P. hohenackeri
P. setaceum
P. squamulatum
P. purpureum
P. glaucum
P. glaucum X P. squamulatum
P. purpureum X P. squamulatum
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

hybrids (lanes 20-22) exhibit zones of activity present in both parents and form intergenic heteropolymers. The P. purpureum X P. squamulatum hybrids (lanes 23-25) are similar to the napiergrass parent indicating the fastest migrating bands in both parents comigrate. APS isozyme phenotypes show extensive variation between Pennisetum species.

The GOT banding patterns (Fig. 19) show comigrating zones of activity present in all the Pennisetum species except P. glaucum for the fastest migrating band. The slowest migrating zone of activity present in the P. purpureum genotypes may comigrate with zones of activity present in the P. glaucum genotypes (lanes 14-19). P. hohenackeri (lane 4) has the same GOT isozyme phenotype as P. purpureum (lanes 11-13). Both of the interspecific hybrid crosses involving P. squamulatum (lanes 20-22 and lanes 23-25) suggest heterozygosity in the P. squamulatum parent. Pearl millet has two GOT loci characterized (Tostain et al. 1987); one of these GOT isozymes may comigrate with a zone of activity present in the P. purpureum phenotypes.

ADH banding patterns were also observed (fig. 20). The three related Pennisetum species, P. squamulatum (lanes 8-10), P. purpureum (lanes 11-13), and P. glaucum (lanes 14-20), have three zones of activity in common. P. purpureum has additional zones of activity as well. P. flaccidum (lanes 2 and 3) has a similar phenotype as P. glaucum. In the P. glaucum X P. squamulatum interspecific hybrids (lanes 21-23) the common

Fig. 19. GOT isozyme banding pattern of Pennisetum species and interspecific hybrids. Protein electrophoresis is from the bottom of the figure towards the top. Leaf extracts were electrophoresed in a starch gel. After electrophoresis the proteins were stained for activity. The order of the samples is P. orientale (PS14) (lane 1), P. flaccidum (PS3) (lane 2), P. flaccidum (PS4) (lane 3), P. hohenackeri (PS156) (lane 4), P. setaceum (PS49) (lane 5), P. setaceum (PS31) (lane 6), P. setaceum (PS22) (lane 7), P. squamulatum (PS24) (lane 8), P. squamulatum (PS26) (lane 9), P. squamulatum (PS158) (lane 10), P. purpureum (PI300086) (lane 11), P. purpureum Merkeron (lane 12), P. purpureum (N14) (lane 13), P. glaucum Tift 23 (lane 14), P. glaucum Tift 18DB (lane 15), P. glaucum Tift 383 (lane 16), P. glaucum Tift 23 (lanes 17, 18, and 19), P. glaucum (176) X P. squamulatum (PS26) (lane 20), P. glaucum (H165) X P. squamulatum (PS26) (lanes 21 and 22), and P. purpureum Merkeron X P. squamulatum (PS262) (lanes 23, 24, and 25).

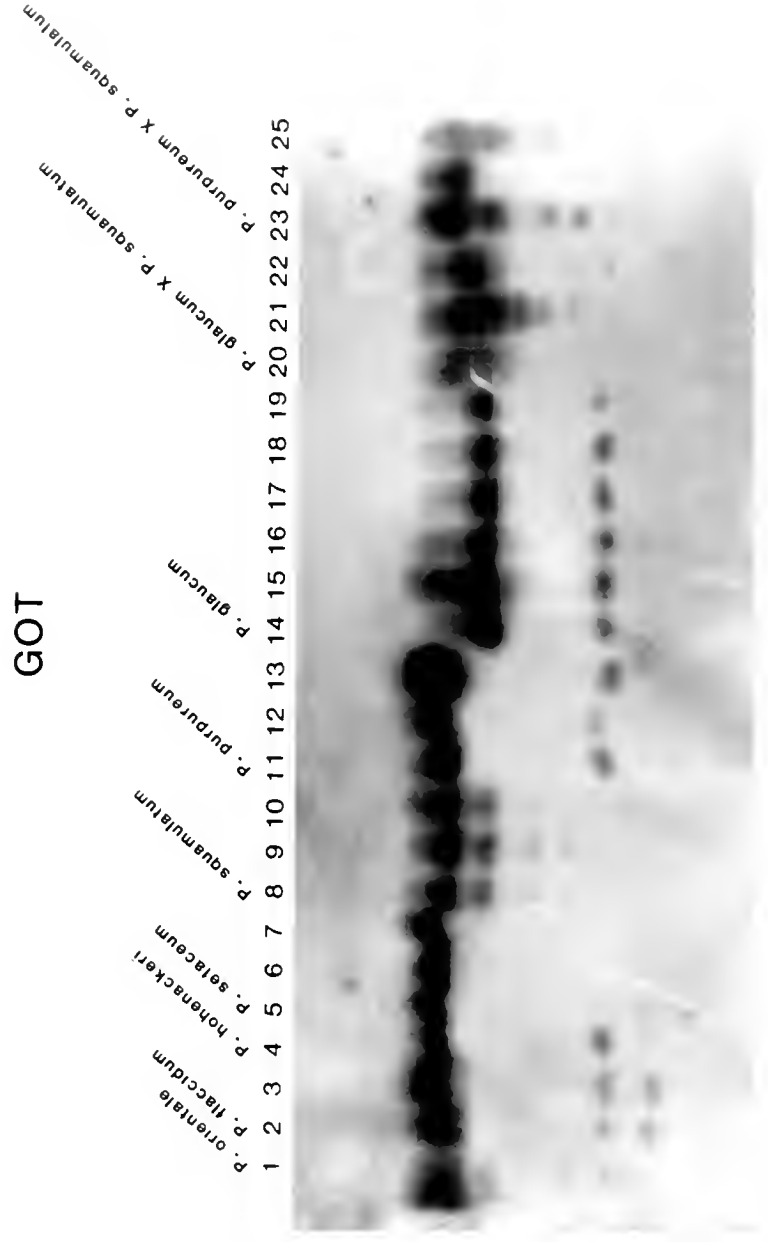
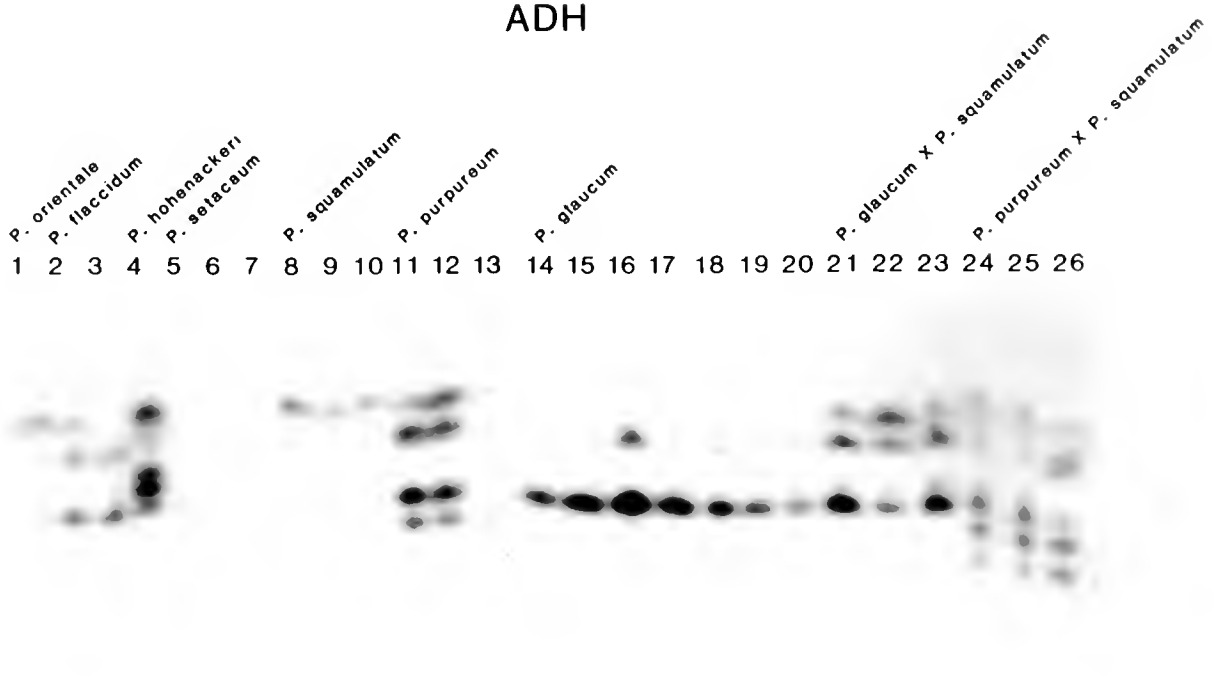

GOT
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
P. orientale
P. flaccidum
P. hohenackeri
P. setaceum
P. squamulatum
P. purpureum
P. glaucum
P. glaucum X P. squamulatum
P. purpureum X P. squamulatum

Fig. 20. ADH isozyme banding pattern of Pennisetum species and interspecific hybrids. Protein electrophoresis is from the bottom of the figure towards the top. Leaf extracts were electrophoresed in a starch gel. After electrophoresis the proteins were stained for activity. The order of the samples is P. orientale (PS14) (lane 1), P. flaccidum (PS3) (lane 2), P. flaccidum (PS4) (lane 3), P. hohenackeri (PS156) (lane 4), P. setaceum (PS49) (lane 5), P. setaceum (PS31) (lane 6), P. setaceum (PS22) (lane 7), P. squamulatum (PS24) (lane 8), P. squamulatum (PS26) (lane 9), P. squamulatum (PS158) (lane 10), P. purpureum (PI300086) (lane 11), P. purpureum Merkeron (lane 12), P. purpureum (N14) (lane 13), P. glaucum Tift 23 (lane 14), P. glaucum Tift 18DB (lane 15), P. glaucum Tift 383 (lane 16), P. glaucum Tift 23 (lanes 17, 18, 19, and 20), P. glaucum (176) X P. squamulatum (PS26) (lane 21), P. glaucum (H165) X P. squamulatum (PS26) (lanes 22 and 23), and P. purpureum Merkeron X P. squamulatum (PS262) (lanes 24, 25, and 26).

ADH

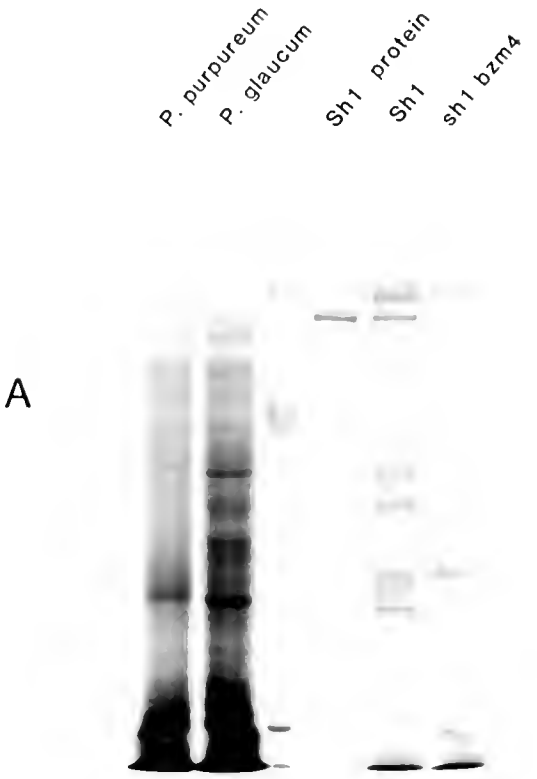

P. orientale
P. flaccidum
P. hohenackeri
P. setaceum
P. squamulatum
P. purpureum
P. glaucum
P. glaucum X P. squamulatum
P. purpureum X P. squamulatum
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26

zones of activity are evident, these likely represent two homodimers and one heterodimer. The P. purpureum X P. squamulatum hybrids (lanes 24-26) have similar phenotypes as the P. purpureum parent and further show the comigration of the three zones of activity common to the parents.

CHAPTER 4
RESULTS OF SUCROSE SYNTHASE COMPARISONS

Pennisetum purpureum and Pennisetum glaucum sucrose synthase subunits were compared to maize sucrose synthase subunits (Fig. 21). Protein was isolated from P. purpureum leaf, P. glaucum seed, maize Sh1 kernel, and maize sh1 bzm4 kernel. These protein samples were electrophoresed in a 7.5% SDS-polyacrylamide gel along with size markers and Sh1 protein from the protein sample used to prepare anti-Sh1 antibodies. The coomassie blue stained protein is shown in Fig. 21A. A heavy staining band of protein in the Sh1 maize lane which comigrates with the Sh1 protein (88,000 mol wt) does not appear in the sh1 bzm4 fractionated protein. A duplicate gel was prepared and the protein was electroblotted to nitrocellulose and probed with anti-Sh1 antibodies. This is shown in Fig. 21B. The sh1 bzm4 protein has a comparatively light anti-Sh1 antibody staining band of 88,000 mol wt; this band is Sus sucrose synthase subunits (Echt and Chourey 1985). The P. glaucum anti-Sh1 antibody staining protein exhibits sucrose synthase degradation due to the isolation technique; however, the darkest staining band is approximately the same size or slightly smaller than the maize Sh1 protein. The darkest antibody staining band present in the P. purpureum

Fig. 21. The sucrose synthase proteins of Pennisetum species. Protein was extracted with 100 mM Tris-HCl (pH 7.5) and 10 mM Dithiothreitol from P. purpureum (PI300086) leaf, P. glaucum Tift 23 seed, Sh1 kernels, and sh1 bzm4 kernels which were frozen and ground in liquid nitrogen. Proteins were electrophoresed on a 7.5% SDS-polyacrylamide gel with the Sh1 protein used to elicit an antibody response and molecular weight markers (A). The Laemmli (1970) buffer system was used. An identical gel was electroblotted onto nitrocellulose. The blotted proteins were reacted with anti-Sh1 antibody (B). Goat anti-Rabbit-alkaline phosphatase conjugate and the color reagents NBT and BCIP from Bio-Rad were used following the suppliers directions.


P. purpureum
P. glaucum
Sh1 protein
Sh1
sh1 bzm4
A


sh1 bzm4
Sh1
Sh1 protein
P. glaucum
P. purpureum
B

protein comigrates with the intact P. glaucum sucrose synthase subunits. This figure shows that the sucrose synthase subunits in these two Pennisetum species are the same size or perhaps slightly smaller than their maize counterparts.

The size of the sucrose synthase enzymes of P. purpureum root tissue and P. glaucum root tissue was compared to maize kernel sucrose synthases (Fig. 22). Isolated protein from the above tissues were electrophoresed in a native polyacrylamide gradient gel, electroblotted onto nitrocellulose, and probed with anti-Sh1 antibodies. The sucrose synthase enzymes from P. glaucum (lane 2) and P. purpureum (lane 3) appear smaller than the maize sucrose synthase enzymes (lane 1). The Pennisetum sucrose synthase enzymes shown in this figure are similar in size to the maize sucrose synthases.

P. purpureum total DNA was probed with a maize Sh1 genomic clone (p17.6) containing the entire structural gene (Fig. 23A) and a maize Sus genomic clone (p21.2) containing exons 3 through 15 (Fig. 23B). Total DNA was digested, electrophoresed, Southern blotted, and probed with the above maize clones. For both A and B, lane 1 is Eco R1 digested P. purpureum DNA; lane 2 is Eco R1 and Bam HI digested DNA; and lane 3 is Eco RI and Hind III digested DNA. The Sh1 probe (Fig. 23A) detects several sequences in the 6 kb to 10 kb region of the Eco RI digest. These restriction fragments include i) sequences which hybridize to the 3' flanking region of the Sh1 structural gene, ii) sequences having similarity

Fig. 22. Fractionated maize, P. glaucum, and P. purpureum proteins stained with anti-Sh1 antibodies. Protein was extracted with 10 mM Tris-HCl (pH 7.2) and 1% mercaptoethanol from maize Sh1 kernel (lane 1), P. glaucum Tift 23 root (lane 2), P. purpureum (PI300086) root (lane 3) which was frozen and ground in liquid nitrogen. Protein was electrophoresed in a 4.5% to 7% linear gradient non-denaturing polyacrylamide gel (Hoefer 1983), electroblotted onto nitrocellulose, and reacted with anti-Sh1 antibodies. Goat anti-Rabbit-alkaline phosphatase conjugate and the color reagents NBT and BCIP from Bio-Rad were used following the suppliers directions.

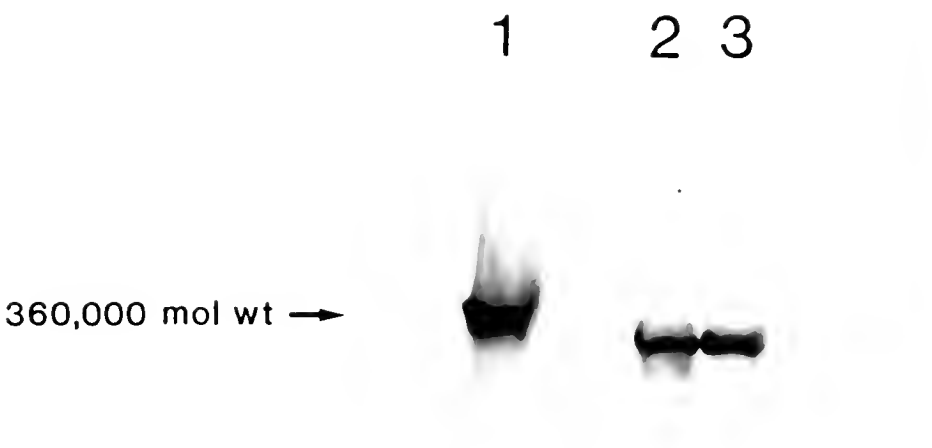
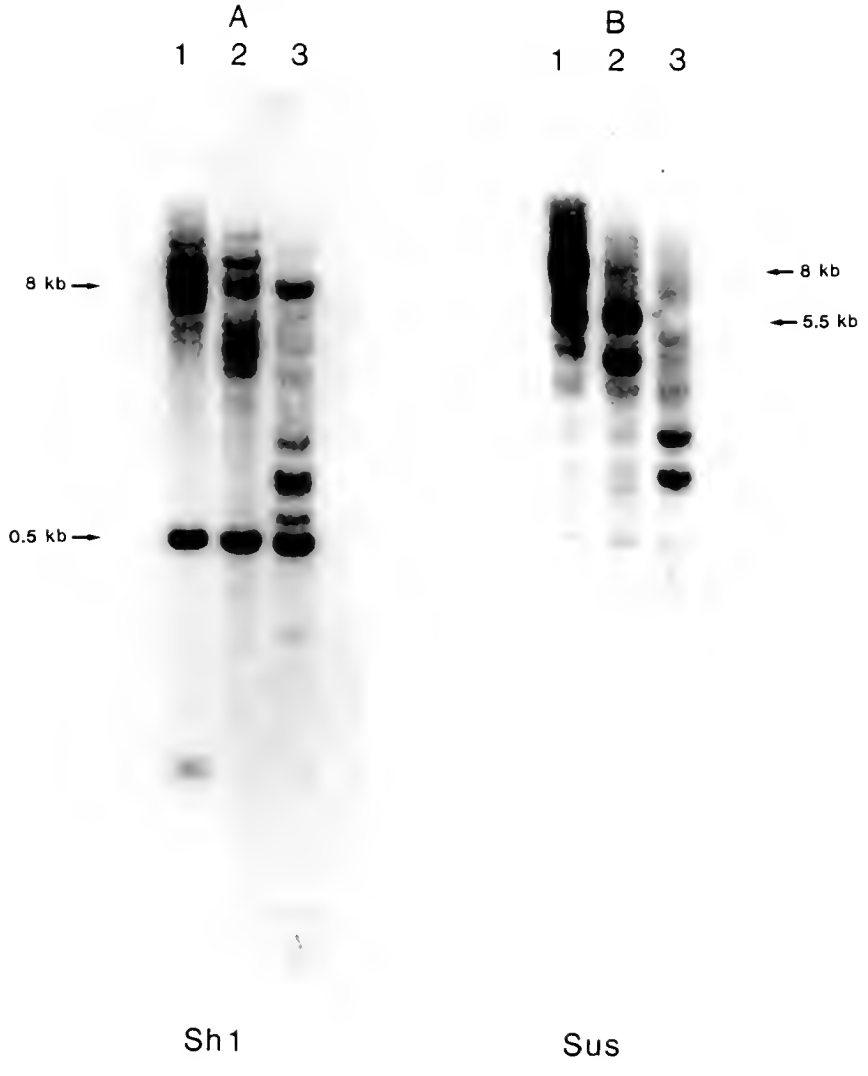

1
2 3
360,000 mol wt

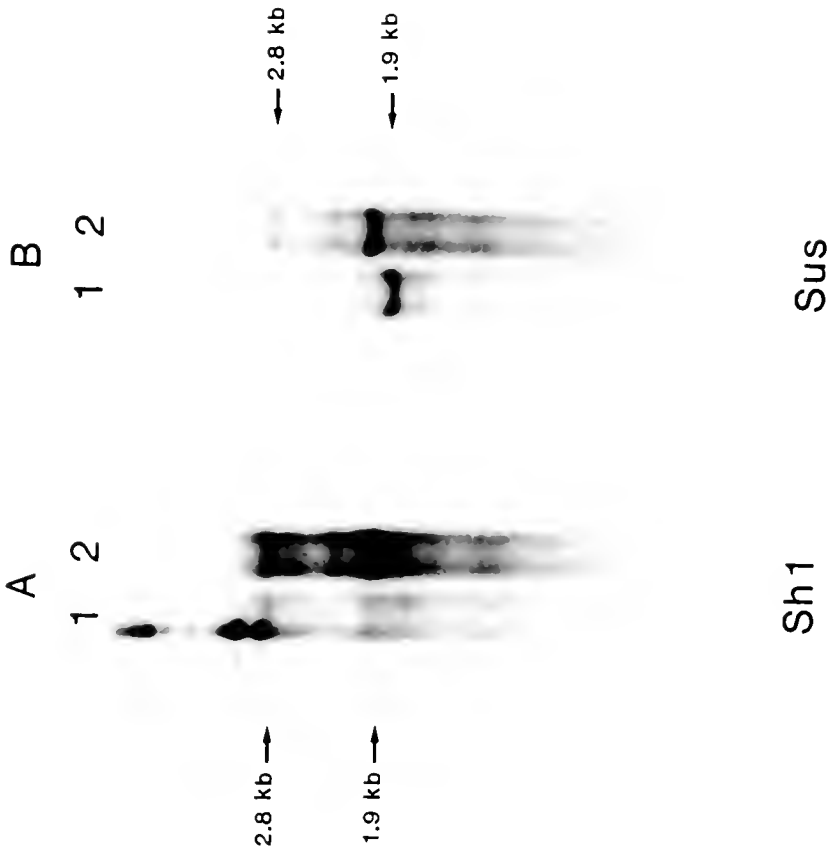
Fig. 23. Cross hybridization of maize sucrose synthase genomic clones Sh1 and Sus to P. purpureum DNA. P. purpureum (PI300086) total DNA (5 ug) was digested with Eco RI (lane 1), Eco RI and Bam HI (lane 2), and Eco RI and Hind III (lane 3). The DNAs were electrophoresed in 0.8% agarose, Southern blotted, probed at 65^0 C with nick translation labeled Sh1 (A) or Sus (B) DNA, and washed at 65^0 C in 0.3X SSPE and 1% SDS.


A
1 2 3
8 kb
0.5 kb
Sh1
B
1 2 3
8 kb
5.5 kb
Sus

to the 5' region of the structural gene including exons 1, 2, 3, and 4, iii) and possibly sucrose synthase genomic sequences which have approximately equal similarity to both Sh1 and Sus such as the structural gene coding for one of the cloned sucrose synthase cDNAs from P. purpureum. The genomic sequence(s) hybridizing the probe most strongly is in the 0.5 kb region. This same sequence(s) may hybridize a P. purpureum sucrose synthase genomic clone containing exons 3 through 15 and the maize gene Sus slightly. The Sus probed P. purpureum DNA has two major bands of hybridization which are 8 kb and 5.5 kb (Fig. 23B, lane 1). Both of these sequences have been cloned and one has been characterized. The 8 kb sequence is the clone p309 described below. Both of these P. purpureum sequences have stronger similarity to Sus as compared to Sh1 as can be seen. It should be noted that in P. purpureum there may be several sucrose synthase genes or non-sucrose synthase sequences which hybridize to the maize probes.

Both P. purpureum leaf poly A^+ RNA and P. glaucum seed poly A^+ RNA were probed with the same Sh1 (Fig. 24A) and Sus (Fig. 24B) probes used in the preceding figure. The Sh1 probe detects a 2.8 kb RNA in both Pennisetum species with the P. glaucum hybridizing more probe in that size region. The maize Sh1 transcript is 2.7 kb in length while the maize Sus transcript is about 2.9 kb in length. The 2.8 kb size range of the P. purpureum and P. glaucum RNAs which hybridize the Sh1 probe likely represent sucrose synthase transcripts. There are

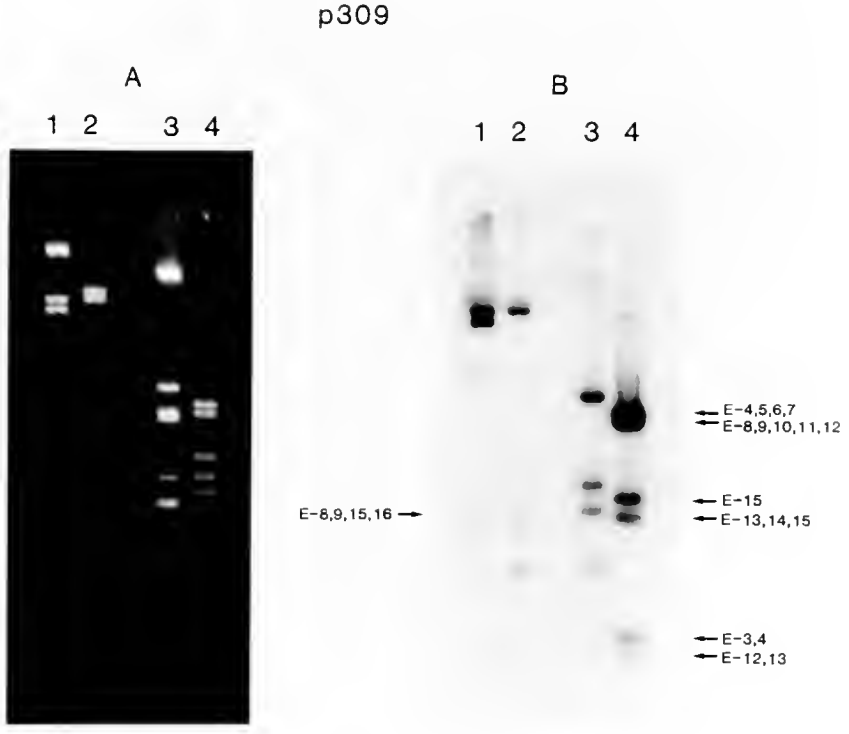
Fig. 24. Cross hybridization of maize sucrose synthase genomic clones Sh1 and Sus to P. purpureum leaf poly A^+ RNA and P. glaucum seed poly A^+ RNA. RNA was isolated from P. purpureum (PI300086) (lane 1) and P. glaucum Tift 23 (lane 2), electrophoresed in 1.2% agarose, blotted, probed at 60^0 C with nick translation labeled Sh1 (A) or Sus (B) DNA, and washed at 60^0 C in 0.3X SSPE and 0.1% SDS.


A
1 2
2.8 kb
1.9 kb
Sh1
B
1 2
2.8 kb
1.9 kb
Sus

smaller RNAs in the 1.6 kb and 1.9 kb size ranges of P. purpureum RNA which also hybridize the Sh1 probe. In P. glaucum there is hybridization of the probe in the 1.9 kb region as well. These are unexpected since maize does not have transcripts this size which hybridize the Sh1 probe. When Sus is used as a probe to identify similar sequences in P. purpureum there is an approximate 1.9 kb size range which hybridizes this probe. An approximate 2.1 kb size range of P. glaucum RNA hybridizes Sus to a greater extent than does the presumptive sucrose synthase transcripts of 2.8 kb. This analysis shows an important difference between maize and these two Pennisetum species. These two Pennisetum species exhibit the strongest hybridization of the maize sucrose synthase probes to transcripts too small to encode sucrose synthase.

A 5 kb to 10 kb Eco RI library was constructed with lambda gtwes used as the vector. The maize sucrose synthase genomic clones Sh1 and Sus were used as probes. One of the presumptive sucrose synthase genomic clones from P. purpureum, p309, was characterized by cross hybridization to triple restriction endonuclease digestions of Sh1 and Sus genomic clones from maize (Fig. 25). The triple digests were electrophoresed (Fig. 25A), Southern blotted, and hybridized to p309. The resulting autoradiograph is seen in Fig. 25B. The P. purpureum genomic clone p309 hybridized to Sus more strongly. Sus was digested with the restriction endonucleases Hind III, Pst I, and Sst I. The regions of Sus (Fig. 25B, lane

Fig. 25. Cross hybridization of *P. purpureum* sucrose synthase genomic clone p309 with maize sucrose synthase genomic clones *Sh1* and *Sus*. *Sh1* was digested with the restriction endonucleases *Hind* III (lane 1), *Hind* III, *Pst* I, and *Hpa* II (lane 2), and *Hind* III, *Pst* I, and *Bgl* I (lane 3). *Sus* was digested with *Hind* III, *Pst* I, and *Sst* I (lane 4). The DNAs were electrophoresed in 1% agarose (A), Southern blotted, probed at 55^0 C with random primer labeled p309 insert, and washed at 55^0 in 0.3X SSPE and 0.1% SDS (B). Restriction fragments of interest are designated, these are labeled E followed by the exons contained on that fragment.


p309
A
1 2 3 4
B
1 2 3 4
E-8,9,15,16
E-4,5,6,7
E-8,9,10,11,12
E-15
E-13,14,15
E-3,4
E-12,13

4) which hybridized p309 include the following: an 1189 bp restriction fragment containing exons 4, 5, 6, and 7; an 1124 bp restriction fragment containing exons 8, 9, 10, 11, and part of 12; a 690 bp restriction fragment containing most of exon 15; a 609 bp restriction fragment containing part of exon 13, exon 14 and a small part of exon 15; a 275 bp restriction fragment containing part of exon 3 and part of exon 4; and a 235 bp restriction fragment containing part of exon 12 and part of exon 13. The triple digest of Sh1 (Fig. 25B, lane 3) hybridized p309 to a lesser extent than it did Sus, lane 4. Sh1 was digested with the restriction endonucleases Hind III, Pst I, and Bgl I. Restriction fragments of Sh1 which hybridize p309 in lane 3 include the following: an 1318 bp fragment containing exons 3, 4, 5, 6, and 7; a 756 bp fragment containing exons 13 and 14; two comigrating restriction fragments of 654 bp containing exons 8 and 9 and 650 bp containing exons 15 and 16; a 455 bp fragment containing exons 10 and 11; and a 262 bp fragment containing exon 12. The genomic clone p309 did not hybridize to the 3' flanking region of Sh1. Different restriction digests were utilized to determine if both the 654 bp and 650 bp fragments hybridized the p309 probe. To determine if exons 8 and 9 of Sh1 hybridize p309, Sh1 was digested with Hind III, Pst I, and Hpa II to yield a 654 bp restriction fragment (lane 2). This fragment containing exons 8 and 9 hybridized p309. When Sh1 was digested with Hind III (lane 1), the 650 bp fragment

containing exons 15 and 16 was shown to hybridize to p309. The P. purpureum sucrose synthase genomic clone p309 hybridized most strongly to Sus restriction fragments containing exons 3 through 15.

The maize Sh1 sucrose synthase gene has 16 exons (Werr et al. 1985) while the maize Sus gene may have 15 exons (Shaw, McCarty, and Hannah, unpublished). Sus does not have an intron corresponding to intron 15 of Sh1 (Shaw, McCarty, and Hannah, unpublished). The P. purpureum sucrose synthase genomic clone p309 was sequenced in the region showing similarity to the 3' end of exon 15 and the 5' end of exon 16 in Sh1. The same strand of a p309 restriction fragment containing this region was sequenced twice. The 20 bp flanking both sides of the region corresponding to the 3' end of exon 15 and the 5' end of exon 16 of Sh1 are shown in Fig. 26. Both strands of the cDNA clone pc309 were sequenced and a comparison is given below the p309 sequence. Following the p309 and pc309 sequences are the corresponding Sus and Sh1 sequences. The genomic sequence from Sus does not contain intron 15. Sus differs from p309 at bases 23, 29, 37, and 39. The Sh1 sequence in Fig. 26 is interrupted; the exon 15 and exon 16 boundaries juxtaposed. As expected the Sh1 sequence differs from p309 to a greater extent than it does from Sus. The Sh1 sequence varies from p309 at positions 2, 5, 14, 16, 18, 23, 27, 28, 32, 36, and 39. The P. purpureum genomic clone p309 does not contain an intron 15 present in the maize Sh1 gene.

The *P. purpureum* p309 Sucrose Synthase Gene, like the Maize *Sus* Gene, does not Contain an Intron 15 as in the Maize *Sh1* Gene

```
                1         10         20         30         40
                .         .          .          .          .
p309 genomic    CGCTCAAGTACCGCACCATG GCCAGCACGGTGCCGTTGGC

pc309 cDNA      CGCTCAAGTACCGCACCATG GCCAGCACGGTGCCGTTGGC

                          Internal Region of Exon 15
Maize Sus       CGCTCAAGTACCGCACCATG GCGAGCACCGTGCCGTGGCC

                     3' End Exon 15     5' End Exon 16
Maize Sh1       CCCTGAAGTACCGTAGCCTG\GCAAGCCAGGTTCCGCTGTC
```

Fig. 26. The gene structure of the *P. purpureum* sucrose synthase genomic clone p309 is compared to the maize sucrose synthase genomic clones *Sh1* and *Sus*. The regions of the genomic clone p309, the cDNA clone pc309, and *Sus* which corresponds to the 3' end of exon 15 and the 5' end of exon 16 of *Sh1* are compared. One strand of p309 was sequenced twice. Both strands of pc309 were sequenced. Intron 15 of *Sh1* has been deleted from this sequence and the exon termini juxtaposed for sequence comparison.

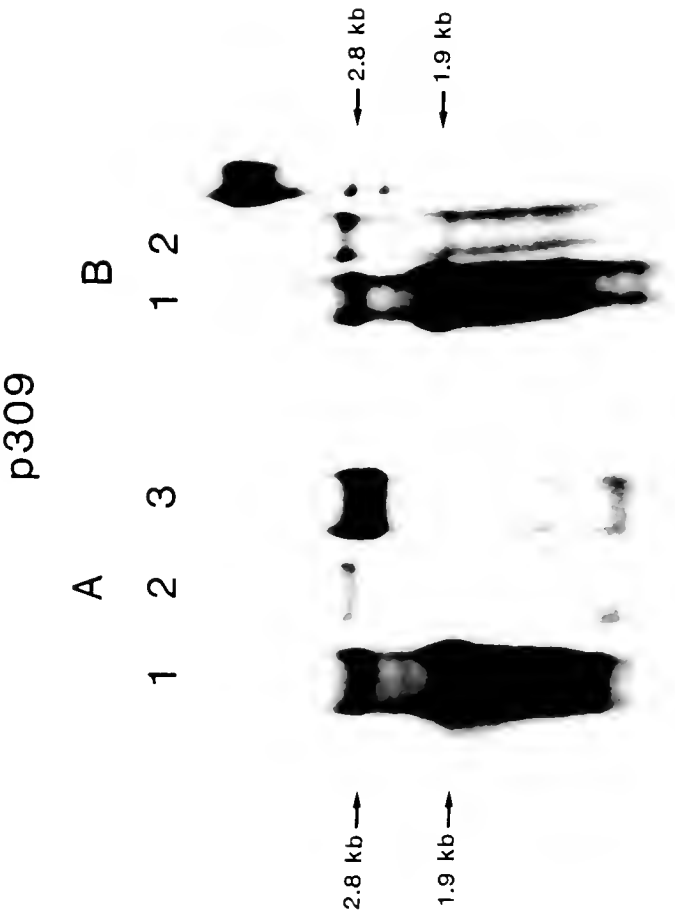
This result was expected because of the close similarity of p309 to the maize sucrose synthase gene Sus.

Intron sequences of p309 were compared to intron sequences of Sus (data not shown). The p309 sequence extended from intron 2 to the 3' end of the structural gene. Different introns exhibited varying degrees of similarity. The sequence comparison of the introns takes into consideration insertions and deletions which have occurred in one gene or the other. Depending on the alignment, the degree of similarity would vary; hence the similarity is an approximation. The last 95 bases of intron 2 of the p309 sequence were the same in 65 out of the 95 bases giving an approximate similarity of 68% between Sus and p309 in this region of the structural genes. This is based upon the sequence of one strand. Both strands of intron 3 were sequenced. Intron 3 in Sus is 90 bases and in p309 it is 89 bases. The similarity was approximately 79% with 71 out of 90 bases identical. One strand of intron 4 was sequenced. Intron 4 in Sus is 125 bases and in p309 it is 115 bases. The approximate similarity was 60% with 75 out of 125 bases the same. Both strands of the 5' end of intron 5 were sequenced. The approximate similarity of the 5' end of intron 5 of p309 to Sus was 62% with 53 out of 85 bases the same. One strand of the 3' end of intron 5 was sequenced. The approximate similarity of the 3' end of intron 5 of p309 to Sus was 60% with 49 out of 82 bases identical. One strand of intron 6 was sequenced. Intron 6 in Sus is 103 bases and in

p309 it is 99 bases. The approximate similarity was 46% with 47 out of 103 bases the same. Both strands of intron 8 were sequenced. Intron 8 in Sus is 90 bases and in p309 it is 79 bases. The approximate similarity was 51% with 46 out of 90 bases the same. Both strands of intron 10 were sequenced. Intron 10 in Sus is 100 bases and in p309 it is 88 bases. The approximate similarity was 52% with 52 out of 100 bases the same; extensive insertions/deletions were present. Both strands of intron 12 were sequenced. Intron 12 in Sus is 92 bases while in p309 it is 87 bases. The approximate similarity was 45% with 41 out of 92 bases the same. One strand including 50 bases of the 5' end of intron 13 was sequenced. The first 3 bases of p309 are the same as the Sus sequence corresponding to that region but other than that the 5' end of intron 13 of p309 appears unrelated to Sus. Both strands of intron 14 were sequenced. Intron 14 in Sus is 77 bases and in p309 it is 93 bases. Intron 14 of p309 appears unrelated to intron 14 of Sus. Surprisingly, the introns in the 5' end of p309 have stronger similarity to the corresponding Sus introns than the introns in the 3' end of the p309 structural gene. Generally, there is a gradient effect of intron similarity between these two structural genes. The progression is from approximately 79% similarity in intron 3 to approximately 50% similarity in the middle introns to no detectable similarity in exons 13 and 14.

RNA was analyzed using p309 as a probe. RNA was isolated, electrophoresed, blotted, and probed. The resulting autoradiograph is shown in Fig. 27. The major transcript size of P. purpureum leaf total RNA which hybridized the probe is the 1.9 kb size range (Fig. 27A, lane 1). The size of the P. purpureum putative sucrose synthase transcript of 2.8 kb was compared to the maize Sus and Sh1 transcripts. The maize deletion mutant sh1 bzm4 kernel poly A^+ RNA is in lane 2 of Fig. 7A. The band of hybridization at 2.9 kb is the Sus transcript. Sh1 mRNA is 200 bases smaller than Sus mRNA. The Sh1 and Sus transcripts from the wild type kernel poly A^+ both hybridized p309 (lane 3). There is considerably more Sh1 mRNA as compared to Sus mRNA in wild type maize kernels; the hybridizational intensity is equal for both suggesting closer similarity of the p309 probe to the Sus transcript. This figure also indicates that the P. purpureum putative sucrose synthase RNA which hybridizes the p309 probe is intermediate in size to the two maize sucrose synthase transcripts. The small maize transcripts hybridizing the probe in lane 3 are about 800 bases and 500 bases in size. The origin of these transcripts is unknown. When P. purpureum leaf poly A^+ RNA is probed with p309 (Fig. 27B, lane 1) the results are the same as the total RNA probed in Fig. 27A, lane 1. P. glaucum seed poly A^+ RNA was also probed with p309 (lane 2); the most intense band of hybridization is in the 2.8 kb size range. The striking result shown in this figure is the RNAs which

Fig. 27. Cross hybridization of P. purpureum sucrose synthase genomic clone p309 with P. purpureum RNA, maize RNA, and P. glaucum RNA. P. purpureum (PI300086) leaf total RNA (lane 1), maize sh1 bzm4 kernel poly A^+ RNA (lane 2) and maize Sh1 kernel poly A^+ RNA (lane 3) were probed (A). Also P. purpureum (PI300086) leaf poly A^+ RNA (lane 1) and P. glaucum Tift 23 seed poly A^+ RNA (lane 2) were probed (B). RNA was isolated, electrophoresed in 1.2% agarose, blotted, probed at 60^0 C, and washed at 60^0 C. The p309 insert probe was labeled by nick translation for A and by random primer for B. The blot in A was washed in 0.3X SSPE and 0.1% SDS; the blot in B was washed in 0.1X SSPE and 0.1% SDS.

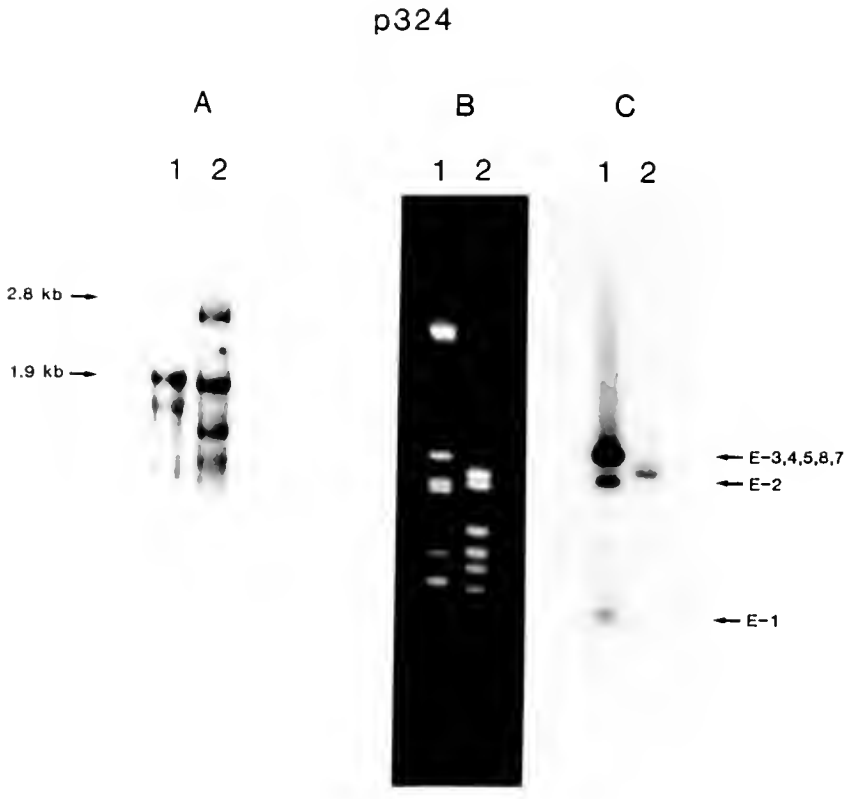

p309
A
1 2 3
B
1 2
2.8 kb
1.9 kb
2.8 kb
1.9 kb

hybridize the probe most strongly in the P. purpureum RNA do not appear to be sucrose synthase mRNAs; these are smaller in size than conventional sucrose synthase mRNA. There is no precedence for this.

One of the clones isolated from the Eco RI library has similarity to the 5' end of the sucrose synthase gene Sh1 from maize. This P. purpureum genomic clone was labeled p324. P. purpureum leaf poly A^+ RNA and P. glaucum seed poly A^+ RNA were electrophoresed, blotted, and probed with p324 (Fig. 28A, lanes 1 and 2 respectively). The major size region of the P. purpureum RNA hybridizing the Sh1 probe is 1.9 kb. A less intense band of hybridization is seen in the 1.6 kb size region. There is a faint band of hybridization seen in the 2.8 kb size region. This hybridization pattern of p324 is similar to the hybridization pattern seen when p309 is used to probe P. purpureum RNA. The P. glaucum RNA hybridized the p324 probe in several size regions as well, these include: the most intense band at around 1.8 kb, a band of hybridization at around 2.5 kb, and another size region of 1.2 kb.

The genomic clone p324 was used to probe triple digests of Sh1 and Sus (Fig. 28, B and C). The digests were electrophoresed (Fig. 28B), Southern blotted, and probed. The resulting autoradiograph is seen in Fig. 28C. Hybridization of p324 to Sh1 was more intense than hybridization to Sus. Restriction endonuclease digestion fragments of Sh1 which hybridized p324 (Fig. 28C, lane 1) include: an 1318 bp

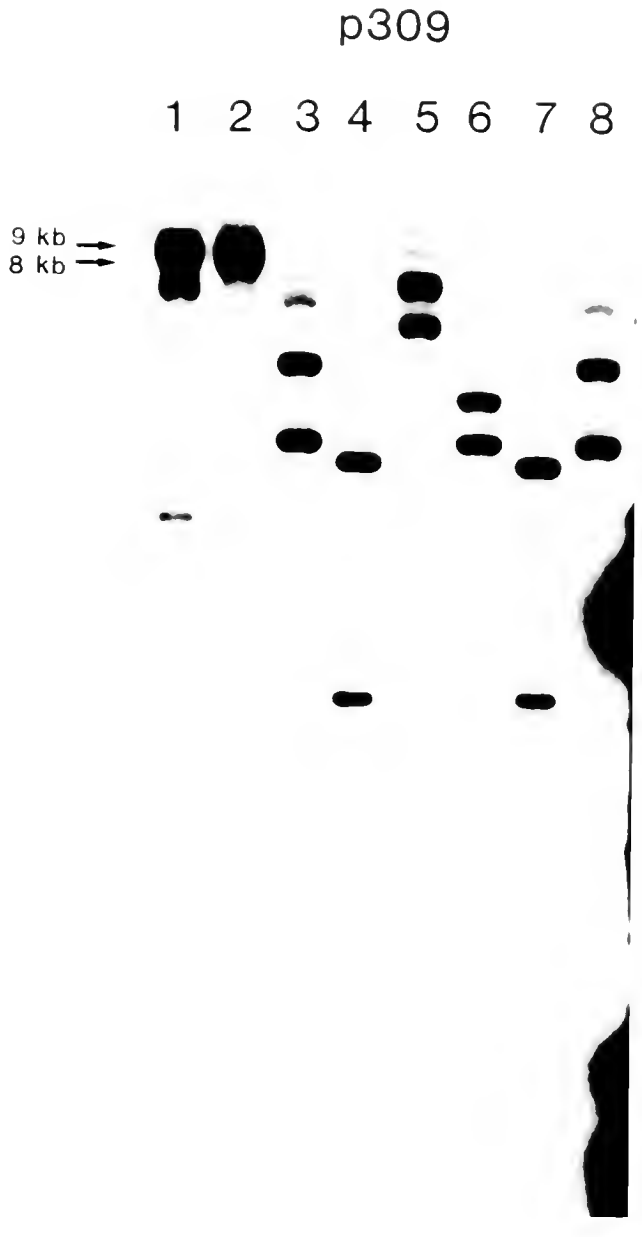
Fig. 28. Cross hybridization of *P. purpureum* genomic clone p324 with *P. purpureum* leaf poly A$^+$ RNA, *P. glaucum* seed poly A$^+$ RNA, and maize sucrose synthase genomic clones *Sh1* and *Sus*. *P. purpureum* (PI300086) RNA (lane 1) and *P. glaucum* Tift 23 RNA (lane 2) were isolated, electrophoresed in 1.2% agarose, blotted, probed at 60^0 C with nick translation labeled p324 insert, and washed at 60^0 C in 0.1X SSPE and 0.1% SDS (A). *Sh1* was digested with the restriction endonucleases *Hind* III, *Pst* I, and *Bgl* I (lane 1). *Sus* was digested with *Hind* III, *Pst* I, and *Sst* I (lane 2). The DNAs were electrophoresed in 1% agarose (B), Southern blotted, probed at 55^0 C with random primer labeled p324 insert, and washed at 55^0 C in 0.3X SSPE and 0.1% SDS (C). Restriction fragments of interest are designated, these are labeled E followed by the exons contained on that fragment.


p324
A
B
C
1 2
1 2
1 2
2.8 kb
1.9 kb
E-3,4,5,8,7
E-2
E-1

fragment containing exons 3, 4, 5, 6, and 7; an 1143 bp fragment containing exon 2; and a 530 bp fragment containing exon 1. Hybridization of p324 to Sus (lane 2) involved a restriction fragment of 1189 bp containing most of exon 4, and exons 5, 6, and 7. Thus, the P. purpureum genomic clone p324 has similarity to the first four exons of sucrose synthase.

Partial sequencing of the P. purpureum genomic clone p309 indicated that the first two exons were not included in the original Eco RI restriction fragment cloned. A cloning strategy which would allow cloning of the 5' end of p309, including at least 1 kb of promotor, as well as the second putative sucrose synthase gene which hybridized Sus (as seen in Fig. 23B) was devised. To develop a strategy P. purpureum total DNA was digested with various restriction endonucleases, electrophoresed, Southern blotted. This blot was hybridized to a 2700 bp restriction fragment from p309 which included exons 6, 7, 8, 9, 10, 11, 12, and most of exon 13. The resulting autoradiograph is seen in Fig. 29. In lane 1 is an Eco RI digest. The restriction fragment represented as p309 is 8 kb in size. The second putative sucrose synthase gene is 5.5 kb in size. When Bam HI is used to digest the P. purpureum DNA (lane 2) the resulting bands of hybridization are 8 kb (this includes the p309 sequence) and 9 kb. From restriction mapping of the p309 Eco RI restriction fragment, and the hybridization pattern with the probe used in Fig. 29, it was determined that the 8 kb Bam HI restriction fragment extended 2.5 kb 5' to the

Fig. 29. Cross hybridization of *P. purpureum* sucrose synthase genomic clone p309 with restriction endonuclease digested *P. purpureum* DNA. *P. purpureum* (PI300086) total DNA (2 ug) was digested with *Eco* RI (lane 1), *Bam* HI (lane 2), *Hind* III (lane 3), *Pst* I (lane 4), *Eco* RI and *Bam* HI (lane 5), *Eco* RI and *Hind* III (lane 6), *Eco* RI and *Pst* I (lane 7), and *Bam* HI and *Hind* III (lane 8). The DNAs were electrophoresed in 0.8% agarose, Southern blotted, probed at 67^0 C with a random primer labeled restriction fragment from p309, and washed at 67^0 C in 0.2X SSPE and 0.1% SDS.

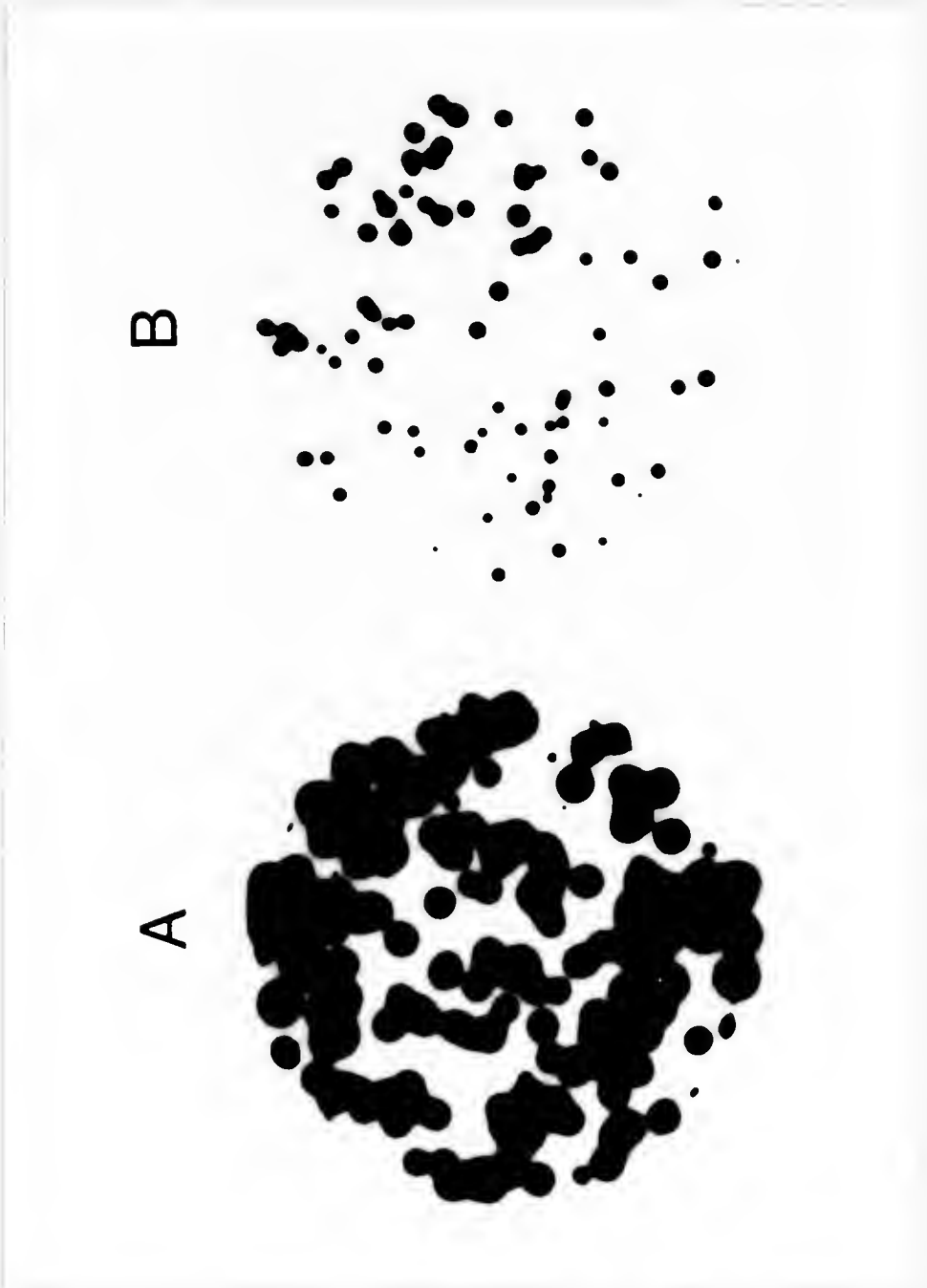

p309
1 2 3 4 5 6 7 8
9 kb
8 kb

original Eco RI clone. To clone these two Bam HI restriction fragments the 7 kb to 11 kb region of Bam HI digested P. purpureum DNA were cloned into the vector EMBL 3.

Approximately 50,000 plaque forming units (pfu) of the 7 kb to 11 kb Bam HI library were screened. The library was plated, lifted onto nylon membrane, and probed with the 2700 bp restriction fragment from p309 containing exons 6 through exons 13. There were two classes of positive clones based upon consistent differences in hybridization intensities. The difference in hybridizational intensity indicated the two sequences of interest having similarity to Sus from maize had been selected from the P. purpureum library. An example of the differential hybridizational intensity is seen in Fig. 30. The DNA from three of the selected Bam HI clones hybridizing the probe more strongly (Fig. 30A) were purified along with the DNA from one of the clones which hybridized the probe with less intensity (Fig. 30B). All three of the putative full length p309 sequences were 8 kb in size while the insert size of p308, a putative sucrose synthase gene, was 9 kb in size. The extent of this project was terminated with the subcloning of these sequences into pUC 19.

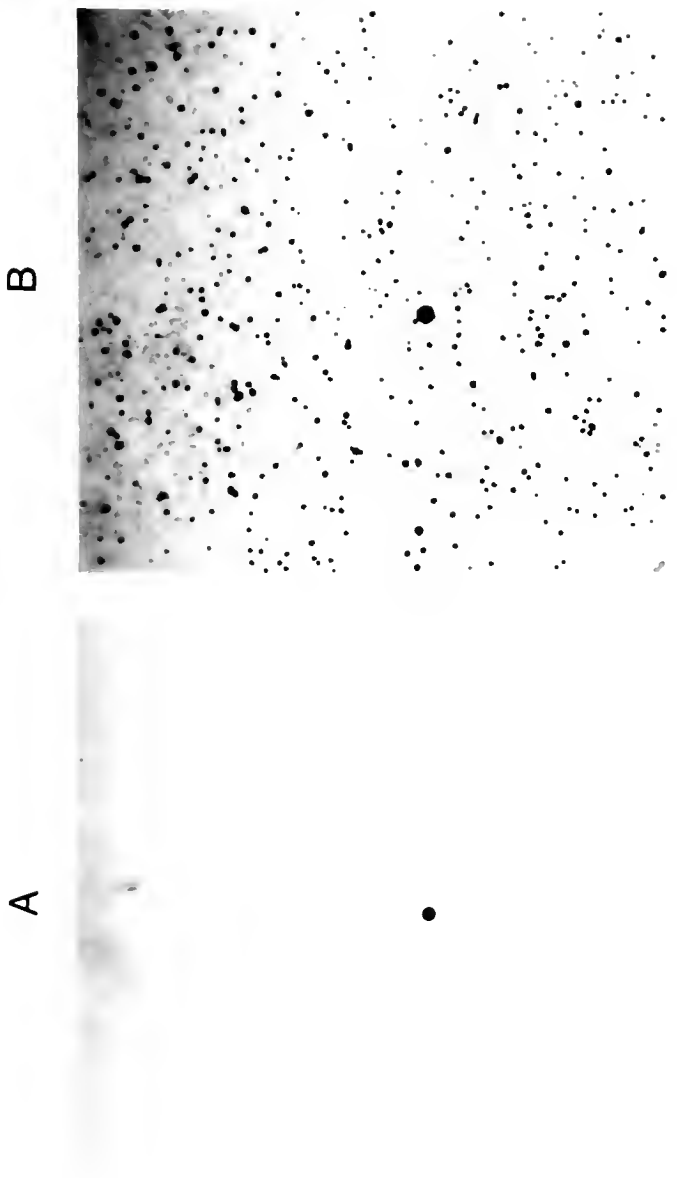
A P. purpureum cDNA library was constructed from leaf poly A^+ RNA. Several clones were selected from this library. To begin the cloning, 300,000 pfu were plated, lifted, and probed. The probe was a 1050 bp restriction fragment from p309 containing exons 14, 15, and 16 along with a 1450 bp

Fig. 30. Cross hybridization of P. purpureum sucrose synthase genomic clone p309 with two plaque-purified EMBL 3 Bam HI genomic clones. P. purpureum (PI300086) genomic clones (A and B) were plated, lifted, probed at 63^0 C with a random primer labeled restriction fragment from p309, and washed at 63^0 C in 0.2X SSPE and 0.1% SDS.


B
A

restriction fragment containing the 3' end of exon 6, exons 7, 8, 9, 10, 11, and most of exon 12. The above probe was used so the cDNAs having similarity to the 5' end of sucrose synthase would not be selected. Four clones were selected from these 300,000 pfu. All four cDNA clones appeared to be from the same locus due to a common restriction digestion pattern but differed in size from 2.6 kb to 2.9 kb. The largest insert was subcloned into pUC 19 and labeled pcNSS. The sucrose synthase cDNA, pcNSS, was determined to not be from the P. purpureum genomic sequence p309 by restriction digest analysis. This conclusion was based upon the prediction of p309 cDNA restriction fragments from Pst I digested cDNA; pcNSS did not have the predicted sizes of Pst I digested cDNA. To select a cDNA coded for by p309 a different strategy was used. The cDNA was size selected by elution of cDNAs greater than 1.8 kb from an agarose gel. This facilitated selection of close to full length sucrose synthase cDNAs along with close to full length cDNAs having similarity to the 5' end of sucrose synthase. About 50,000 pfu containing inserts greater than 1.8 kb in size were screened using a 2700 bp restriction fragment from p309 containing exons 5 through 13. Two sucrose synthase cDNAs were isolated from the above size-fractionated cDNAs. The autoradiography of one of the lifts from the size-fractionated cDNA probed with the p309 restriction fragment is shown in Fig. 31A. One of these two sucrose synthase cDNAs was another pcNSS cDNA while the other was shown to be encoded by

Fig. 31. Cross hybridization of P. purpureum sucrose synthase genomic clone p309 and P. purpureum sucrose synthase cDNA clone pcNSS with the same P. purpureum cDNA clones. Size fractionated cDNA clones were plated, lifted, probed at 65^0 C with a random primer labeled restriction fragment from p309 (A), and washed at 65^0 C in 0.2X SSPE and 0.1% SDS. After autoradiography of the lift it was reprobed using the same conditions with pcNSS insert (B).

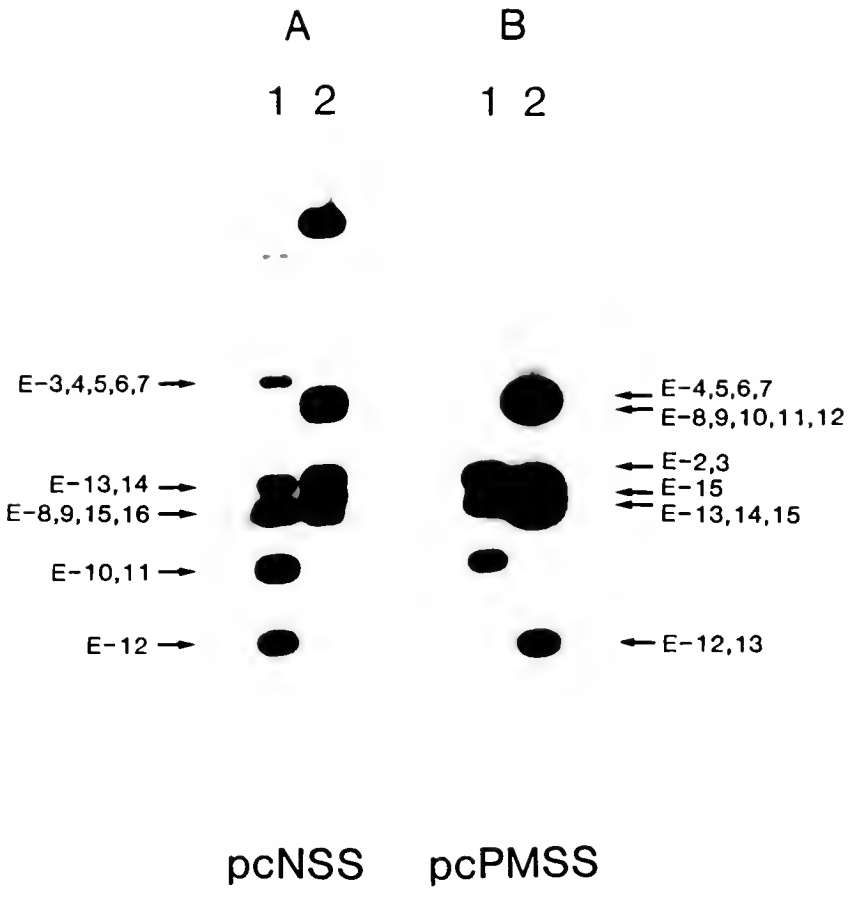

B
A

the p309 genomic clone. This 3 kb p309 cDNA was labeled pc309 and sequenced. The lifts screened with exons 5 through 13 of p309 were rescreened with pcNSS (Fig. 31B). About 350 pfu out of the 25,000 pfu probed in Fig. 31A hybridized the pcNSS probe. This amounted to approximately 1.4% of the cDNA greater than 1.8 kb. These cDNAs likely hybridized to the 5' end of pcNSS since the difference in the two probes, besides the fact that one is a structural gene and the other is a cDNA encoded by another gene, is the cDNA sequence 5' to exon 5 present in pcNSS. It is also likely that the abundant 1.9 kb mRNA which hybridizes to sucrose synthase probes and to p324 is represented in the cDNAs shown in Fig. 31B. Several of the cDNAs representing 1.4% of the size fractionated cDNAs were isolated and may be investigated at a later date.

A P. glaucum cDNA library was constructed from seed poly A^+ RNA. Approximately 150,000 pfu from the library were plated, lifted, and screened. The probe was a p309 restriction fragment containing the 3' end of exon 6, exons 7 through 11, and most of exon 12. Four cDNAs which preferentially hybridized the probe were selected from the library. They were subcloned into pUC 19. These four P. glaucum cDNAs were from 1.5 kb to 2.6 kb in size. One of the four had an internal Eco RI site indicating at least two populations of cDNAs. The largest cDNA was selected for further investigation. This cDNA was labeled pcPMSS and sequenced.

Two sucrose synthase cDNA clones, pcNSS from P. purpureum and pcPMSS from P. glaucum, were used to probe triple digests of Sh1 and Sus. The DNA was digested, electrophoresed, Southern blotted, and probed. The resulting autoradiographs are shown in Fig. 32. Restriction fragments from the Sh1 triple digest in lane 1 of both Fig. 32A and Fig. 32B are labeled on the left side of the figure. Restriction fragments from the Sus triple digest in lane 2 of both Fig. 32A and Fig. 32B are labeled on the right side of the figure. The cDNA clone pcNSS hybridizes with different intensity to the 5' and 3' regions of Sh1 (Fig. 12A, lane 1). The Sh1 restriction fragment containing exons 3 through 7 exhibits weak hybridization of pcNSS when compared to the other Sh1 restriction fragments containing coding sequences which hybridize to pcNSS. Neither of the restriction fragments of Sh1 containing exon 1 or exon 2 hybridized the pcNSS probe. The two restriction fragments of Sh1 which contain exons 8 and 9 or exons 15 and 16 comigrate, hence their individual hybridization patterns are unknown. The restriction fragments containing exons 10 and 11 along with exon 12 both hybridize pcNSS strongly when compared to the 5' end of the maize Sh1 genomic sequence. The restriction fragment containing exons 13 and 14 hybridizes the probe moderately. The similarity of pcNSS to the 5' end of Sus (lane 2) is much stronger than to Sh1. All of the Sus restriction fragments which hybridize pcNSS do so with similar intensity. The Sus restriction

Fig. 32. Cross hybridization of P. purpureum sucrose synthase cDNA clone pcNSS and P. glaucum sucrose synthase cDNA clone pcPMSS with maize sucrose synthase genomic clones Sh1 and Sus. Sh1 was digested with the restriction endonucleases Hind III, Pst I, and Bgl II (lane 1). Sus was digested with Hind III, Pst I, and Sst I. The DNAs were electrophoresed in 1.2% agarose, Southern blotted, probed at 65^0 C with pcNSS (A) or probed at 63^0 C with pcPMSS insert (B), and washed at the hybridization temperatures in 0.3X SSPE and 0.1% SDS. Restriction fragments of interest are designated, these are labeled E followed by the exons contained on that fragment. The slowest migrating restriction fragments which hybridized the pcNSS probe (A) in both lanes 1 and 2 contain the pBR322 vector; this is vector on vector hybridization.


A
B
1 2
1 2
E-3,4,5,6,7
E-13,14
E-8,9,15,16
E-10,11
E-12
E-4,5,6,7
E-8,9,10,11,12
E-2,3
E-15
E-13,14,15
E-12,13
pcNSS
pcPMSS

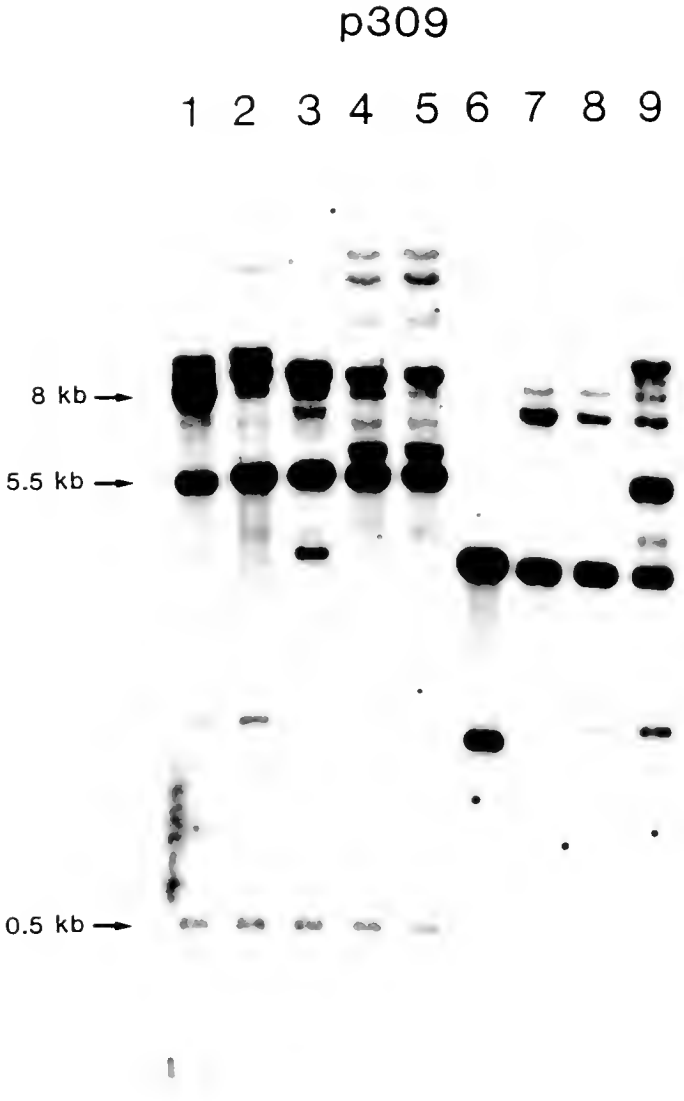
fragment of 874 bp containing part of exon 2 and the 5' end of exon 3 hybridizes pcNSS. The Sus restriction fragment containing most of exon 4 and exons 5, 6, and 7 hybridizes the probe with more intensity than the Sh1 restriction fragment containing exons 3 through 7. The Sus restriction fragment containing exons 8 through 11 and part of exon 12 hybridizes pcNSS. But, the restriction fragment containing the 3' end of exon 12 and the 5' end of exon 13 does not hybridize to the probe. There is hybridization of pcNSS to the Sus restriction fragments which contain exons 13, 14, and 15. The P. purpureum sucrose synthase cDNA clone pcNSS does not fit into a maize Sh1 or Sus classification as p309 or pcPMSS does.

The P. glaucum sucrose synthase cDNA clone pcPMSS has more similarity to Sus of maize than to Sh1 (Fig. 32B). Sequencing has shown this cDNA clone contains part of exon 5 and the rest of the downstream coding region. However, pcPMSS does not hybridize to the Sh1 restriction fragment containing exons 3, 4, 5, 6, and 7 (lane 1). There is moderate hybridization to the two comigrating restriction fragments containing exons 8 and 9 and 15 and 16. The Sh1 restriction fragments containing exons 10 and 11 as well as 13 and 14 also hybridize pcPMSS moderately. There is very weak hybridizational intensity of the probe to the restriction fragment containing exon 12. Probing Sus with pcPMSS exhibits a strong similarity between these two sequences (lane 2). As expected, there is no hybridization of the probe to the

restriction fragment containing regions of exons 2 and 3. There is strong hybridizational intensity of pcPMSS to Sus when compared to the rest of the figure. The rest of the Sus restriction fragments hybridizing pcPMSS hybridize strongly except for the restriction fragment containing parts of exons 12 and 13. The close similarity of pcPMSS to Sus mimics the similarity of p309 to Sus.

A restriction fragment of the P. purpureum genomic clone p309 was used to probe several napiergrass DNAs, two P. squamulatum DNAs, P. hohenackeri, two P. glaucum DNAs, and a P. glaucum X P. purpureum interspecific hybrid. The restriction fragment contained exons 5 through 13. The DNAs were digested with the restriction endonuclease Eco RI, electrophoresed, Southern blotted, and probed. The resulting autoradiograph is shown in Fig. 33. The P. purpureum from which the clone p309 was isolated is in lane 1. There are two major bands of hybridization in lane 1. The 8 kb band represents the p309 sequence. The 5.5 kb band is the sequence putatively cloned and termed p308. The two bands which migrate as restriction fragments larger than 8 kb may be partial digests. When the same DNA is digested with Bam HI only two bands are visible suggesting that the two bands of hybridization larger than 8 kb present in lane 1 are partial digest. The band of hybridization in the 0.5 kb region is of unknown origin. As can be seen P. squamulatum appears to contain sequences in common with the P. purpureum DNAs (lanes

Fig. 33. Cross hybridization of *P. purpureum* sucrose synthase genomic clone p309 with selected *Pennisetum* species. *P. purpureum* (PI300086) (lane 1), *P. purpureum* (N16) (lane 2), *P. purpureum* (N137) (lane 3), *P. squamulatum* (PS24) (lane 4), *P. squamulatum* (PS26) (lane 5), *P. hohenackeri* (PS156) (lane 6), *P. glaucum* Tift 23 (lane 7), *P. glaucum* Tift 23A (lane 8), and *P. glaucum* Tift 23A X *P. purpureum* (N16) total DNAs (2 ug) were digested with *Eco* RI. The DNAs were electrophoresed in 0.8% agarose, Southern blotted, probed at 68^0 C with a random primer labeled restriction fragment from p309, and washed at 68^0 C in 0.1X SSPE and 0.1% SDS.

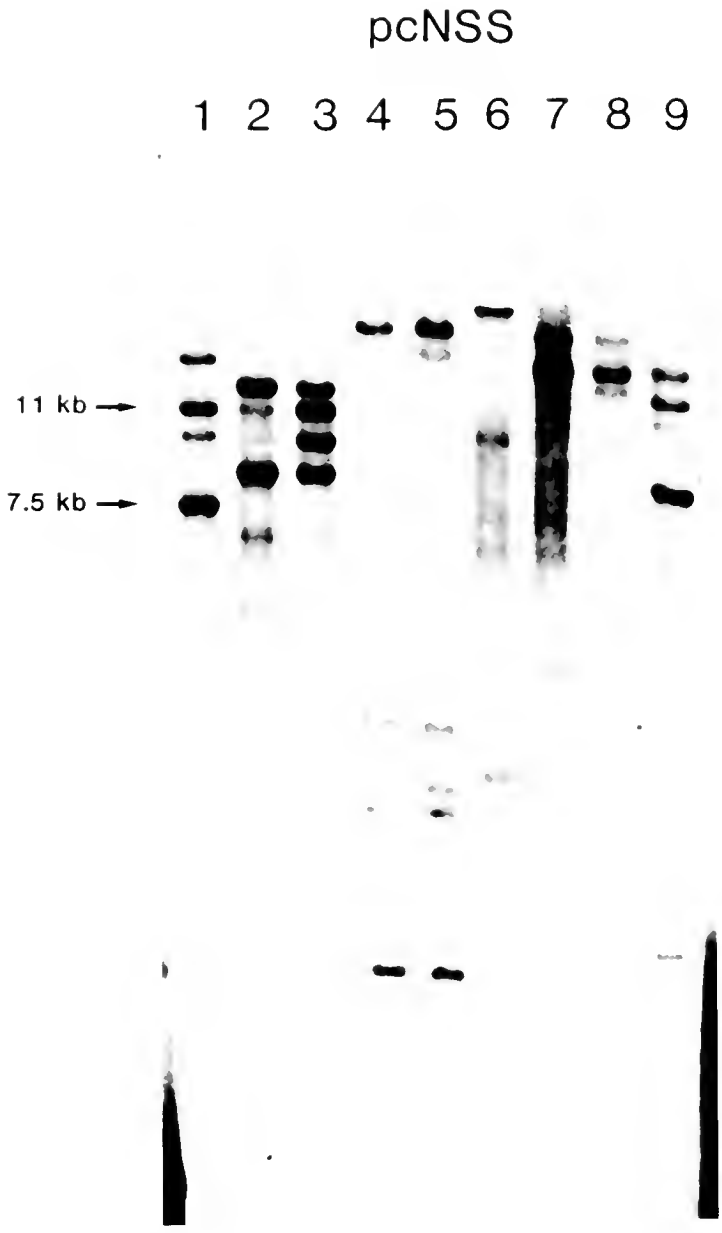

p309
1 2 3 4 5 6 7 8 9
8 kb
5.5 kb
0.5 kb

4 and 5). Oddly enough, the strongest band of hybridization is seen with P. hohenackeri DNA. The two P. glaucum DNAs (lanes 7 and 8) show one major band of hybridization. As expected, the triploid in lane 9 contains bands of hybridization from both parents. These bands are considered to be Pennisetum sucrose synthase genes similar to the maize Sus sucrose synthase gene.

An identical Southern blot was probed with the P. purpureum cDNA sucrose synthase clone pcNSS (Fig. 34). The restriction fragments which cross hybridize to this probe do not cross hybridize to p309 on Southern blots. There are two major bands of hybridization in the P. purpureum DNA (lane 1) of 11 kb and 7.5 kb. It is unknown which is the pcNSS coding sequence. The bands of hybridization detected with the pcNSS probe in all Pennisetum species differ from the bands with p309. The data are interpreted as indicating that there is more than one sucrose synthase coding locus in these species. This interpretation is suggestive of what is seen in maize, a dual sucrose synthase system.

The DNA sequence of the P. purpureum sucrose synthase cDNA clone was determined as well as the sequence of the P. glaucum sucrose synthase cDNA clone pcPMSS (Fig. 35). Both strands of both clones were sequenced. The clone pc309 is 2949 bp in length excluding a poly A^+ tail of approximately 80 bases. This is likely a nearly full length cDNA clone. The putative exon boundaries are indicated; the position of the

Fig. 34. Cross hybridization of *P. purpureum* sucrose synthase genomic clone p309 with selected *Pennisetum* species. *P. purpureum* (PI300086) (lane 1), *P. purpureum* (N16) (lane 2), *P. purpureum* (N137) (lane 3), *P. squamulatum* (PS24) (lane 4), *P. squamulatum* (PS26) (lane 5), *P. hohenackeri* (PS156) (lane 6), *P. glaucum* Tift 23 (lane 7), *P. glaucum* Tift 23A (lane 8), and *P. glaucum* Tift 23A X *P. purpureum* (N16) total DNAs (2 ug) were digested with *Eco* RI. The DNAs were electrophoresed in 0.8% agarose, Southern blotted, probed at 67^0 C with random primer labeled pcNSS insert, and washed at 67^0 C in 0.1X SSPE and 0.1% SDS.


pcNSS
1 2 3 4 5 6 7 8 9
11 kb
7.5 kb

DNA Sequence of pc309 and pcPMSS

```
1
CCTCCGTTCA CCCCGTCCAT TTGATTTGCG TTTAGTGCGT TACGTTTCTT

51
GAGTTCCCCC TCTTTGGAAG GAGCATCTCC TTCCTGTTCT CCTCTCGGAG

101
AAAGGCTTGA GGACCCAAGA AGAGGATATC AATGGGGGAA GCTGCCGGCG

151
ACCGTGTGCT GAGCCGCCTC CACAGCGTCA GGGAGCGCAT CGGCGACTCC

201                                                  E3
CTCTCCGCTC ACCCCAATGA GCTCGTCGCC GTCTTCGCCA GGCTGAAAAA

251
CCTTGGAAAG GGCATGCTGC AGCCCCACCA GATCATTGCT GAGTACAACA

301
GCGCTATCCC TGAAGCTGAG CGCGAGAAGC TGAAGGATGG TGCCTTTGAG

351                     E4
GATGTCCTGA GGGCAGCTCA GGAAGCAATT GTTATCCCCC CATGGGTTGC

401
ACTTGCCATC CGCCCCAGGC CTGGTGTCTG GGAGTATGTG AGGGTCAATG

451
TCAGCGAGCT CGCTGTTGAG GAGCTGAGAG TCCCTGAGTA CCTGCAGTTC
```

Fig. 35. The DNA sequence of the P. purpureum sucrose synthase cDNA clone pc309 compared to the DNA sequence of the P. glaucum cDNA clone pcPMSS. The 2950 base DNA sequence of pc309 is shown. Beneath the pc309 sequence are the bases of pcPMSS which differ from the pc309 sequence. Dots beneath the pc309 sequence indicate the same base at the same position in pcPMSS as compared to pc309. At bases 132 and 217 possible start translation codons are underlined. At base 2749 a possible stop translation codon is underlined. At base 2896 the putative add poly A signal is underlined. Possible exons have been indicated by an E directly above the start of the exon followed by the number of the individual exon. The putative exon boundaries were determined from the maize Sus gene structure.

```
501                          E5
AAGGAACAGC TTGTGGAAGA AGGCCCCAAC AACAACTTTG TTCTTGAGCT

551
GGACTTTGAG CCATTCAATG CGTCCTTCCC CCGTCCTTCT CTGTCCAAGT

601
CCATTGACAA TGGTGTGCAG TTCCTCAACA GGCACCTGTC GTCAAAGCTG
......G... .......... .......... .....T.... ..........

651
TTCCATGACA AGGAGAGCAT GTACCCCCTG CTCAACTTCC TTCGTGCCCA
.......... .......... .......... .......... ..........

701               E6
CAACTACAAG GGCATGACCA TGATGTTGAA CGACAGAATC CGCAGTCTCA
.......... .......... .......... .......... ..T.......

751
GTGCTCTCCA AGCCGCTCTG AGGAAGGCTG AGGAGCATCT GTCCAGCCTT
.......... .......... .......... .......... ..........

801                                    E7
CCAGCTGATA CTCCATACTC TGACTTCCAC CACAGGTTCC AGGAACTTGG
.......... .C........ .......... .......... ..........

851
TCTGGAGAAG GGTTGGGGTG ACTGCGCTAA GCGTTCCCAG GAGACTATTC
.......... .......... .......... .......... ..........

901
ACCTCCTCTT GGACCTTCTT GAGGCCCCTG ATCCGTCCAC CCTGGAGAAG
.......... .......... .......... .......... ..........

951
TTCCTTGGGA CGATCCCCAT GGTGTTCAAT GTTGTTATCC TGTCCCCTCA
.......... .A........ .......... .......... ..........

1001
TGGTTACTTT GCTCAAGCTA ATGTGTTGGG TTACCCTGAC ACTGGAGGCC
.......... .......T.. .......... .......... ..........
 E8
1051
AGGTTGTGTA CATTTTGGAT CAAGTCCGTG CTATGGAGAA TGAAATGCTG
.......... .......... .......... .......... ..........

1101                                                   E9
CTGAGGATCA AGCAGTGCGG TCTTGACATC ACACCAAAGA TCCTTATCGT
.......... .......... .......... .......... ..........
```

Fig. 35. (continued)

```
1151
CACCAGGTTG CTCCCTGATG CAACTGGCAC CACCTGTGGC CAGCGTCTTG
.......... .......... .......... .......... ..........

1201
AGAAGGTCCT TGGCACTGAG CACTGCCATA TCCTTCGTGT CCCATTCAGA
.......... .......... .......... .......... ..........

1251
ACTGAGAATG GAATTGTTCG CAAGTGGATC TCGCGTTTTG AAGTCTGGCC
.......... .......... .......... .......... ..........

1301                      E10
ATACCTGGAG ACTTACACCG ATGATGTGGC ACACGAGATT GCTGGAGAAC
.......... .......... .......... .......... ..........

1351
TCCAGGCCAA TCCTGACCTG ATCATTGGAA ACTACAGTGA TGGAAACCTT
.......... C......... .......... .......... ..........

1401                                        E11
GTTGCTTGTT TGCTTGCCCA CAAGATGGGT GTTACTCACT GTACCATTGC
.......... .......... .......... .........T. ..........

1451
CCATGCCCTT GAGAAAACTA AGTACCCTAA CTCTGACCTC TACTGGAAGA
T......... .......... .......... .......... ..........

1501
AGTTCGAGGA CCACTACCAC TTCTCGTGCC AATTCACCAC TGACTTGATT
.......... .......... .......... .......... ..........

1551
GCCATGAACC ACGCCGACTT CATCATCACC AGTACCTTCC AAGAGATTGC
.......... .......... .......... .......... ..........

1601  E12
TGGAAACAAG GACACTGTTG GCCAGTATGA GTCCCACATG GCCTTCACAA
.......... .......... .T........ .......... ..........

1651
TGCCTGGTCT GTACCGCGTT GTCCACGGTA TCGATGTGTT TGACCCTAAG
.......... .......... .......... .......... ..........

1701
TTCAACATTG TCTCGCCTGG TGCGGACATG TCCATCTACT TCCCTTACAC
.......... .......... .......... .......... ..........
```

Fig. 35. (continued)

```
1751
CGAGTCACAC AAGAGGTTGA CCTCCCTCCA CCCTGAGATT GAGGAGCTCC
.......... .......... ....T..... .......... ..........

1801                                  E13
TCTACAGCCA AACCGAGAAC AACGAGCACA AGTTTGTGCT GAACGACAGG
.......... .......... .......... .......... ..........

1851
AACAAGCCCA TCATCTTCTC CATGGCTCGT CTTGACCGTG TCAAGAACTT
.......... .......... .......... .........C. ..........

1901
GACTGGTCTG GTTGAGCTGT ACGGCCGGAA CAAGCGCCTG CAGGAGTTGG
.......... .......... .......... .......... ..........

1951
TTAACCTTGT GGTTGTCTGC GGTGATCACG GCAACCCGTC CAAGGACAAG
.......... .......... .....C.... .......... ..........

2001
GAGGAGCAGG CTGAGTTCAA GAAGATGTTT GACCTCATCG AGCAGTACAA
.......... .......... .......... .......... ..........

2051
CCTGAACGGG CACATCCGCT GGATCTCTGC CCAGATGAAC CGTGTCCGCA
.......... .......... .......... .......... ..........

2101
ACGCTGAGCT CTACCGCTAC ATCTGCGACA CCCAGGGTGC CTTCGTGCAG
.......... .......... .......... .......... ..........
E14
2151
CCTGCTTTCT ACGAGGCTTT CGGTCTGACT GTGGTTGAGG CCATGACCTG
.......... .......... .......... .......... ..........

2201
CGGTCTGCCC ACATTCGCTA CCGCCTACGG TGGTCCAGCT GAGATCATCG
.......... .......... .......... .......... ..........

2251
TGCACGGCGT GTCCGGCTAC CACATTGACC CGTACCAGGG CGACAAGGCC
.......... .......... .......... .......... ..........

2301
TCGGAGCTGC TCGTGGACTT CTTTGAGAAG TGCAAGGAGG ATTCCAGCCA
.......... .......... .......... .......... ..........
```

Fig. 35. (continued)

```
2351                                                          E15
CTGGAGCAAG ATCTCCCAGG GCGGGCTCCA GCGTATCGAG GAGAAATACA
.......... ....T..... .......... .......... ..........

2401
CCTGGAAGCT CTACTCGGAG AGGCTGATGA CCCTGACCGG CGTTTACGGG
.A........ .......... .......... .......... ..........

2451
TTCTGGAAGT ACGTGTCCAA CCTGGAGAGG CGCGAGACCC GGCGGTACCT
.......... .......... .......... .......... ..........

2501
GGAGATGCTG TACGCGCTCA AGTACCGCAC CATGGCCAGC ACGGTGCCGT
.......... .......... .......... .......... ..........

2551
TGGCCGTGGA GGGAGAGCCC TCCAGCAAGT GATGCGGCCG CCTGGCCGGG
.......... .......... .......... .......... ...-------

2601
GCCTGAAAGA AGGGAGCACT TGAAGTCTGT GTCTTTTCCT GGGTTCCTTC
----A..... .......... .......... .......... ..........

2651
CTAATTTCCT CCCCCTGATT CGGGGGTTCA GAGGCGAAAC AAAGAGAGTT
.......... .......... .......... .......... ..........

2701
TGGTTTTTCC TAGGCGGGCA TCGTCGCTGC TCTTTCTTCT TCAAGAATTA
.......... .......... .......... .......... ..........

2751
AGGTCTTCCA TTGATGTGGG AGTTGCTGCT AGTTTGAGTT GATTTAGTAG
.......... .......... .......... .......... ..........

2801
TTGGGGATGG ATGGGGTTGG GGGGTGTGTG TGTGCAGTCG GTCATGGGTG
......G... .......... .......... .......... ...G...-..

2851
CTCCCTTAGC TTGTTTCCTG GATGGATGGA TGGATGTGCT CCTTCAATAA
.......... .......... .......... .......... ..........

2901
ACGCAGCGGT CTTGGTTGCC GTTTCTGAAA TAAGAGTAGC ATTCATCTCA
.........C .......... .......... .......... ........TT
```

Fig. 35. (continued)

2951
Poly A^+
Sequence continues for 241 bases until poly A^+

Fig. 35. (continued)

exon 1/2 boundary is unknown because the gene structure of the 5' end of the maize Sus gene and the P. purpureum genomic clone p309 are unresolved. The P. glaucum clone pcPMSS extends from the middle of exon 5 past the 3' end of the pc309. The 3' end of pcPMSS extends 242 bp past the polyadenylation site of pc309. This extension may be a cDNA cloning artifact; the origin of this extension is unknown.

There are several possible signal sequences indicated in Fig. 35. Two possible ATG start translation codons are at the 5' end of the pc309 sequence. The first ATG at base 132 is separated by 5 bases from the tetramer AGGA. This is characteristic of the procaryotic start translation Shine-Dalgarno sequence (Shine and Dalgarno 1975) found in E. coli. The tetramer AGGA in E. coli potentially base pairs with the 3' end of the 16s ribosomal subunit. The second ATG at base 217 is more similar to a eucaryotic start translation consensus sequence CCA/GCCATGG (Kozak 1985). The second ATG is preceded by CCCCA. The maize Sus and Sh1 cDNAs used in the sequence comparison to pc309 also contain two ATG codons. The same flanking sequences are present in both of these ATG sequences in Sus. Characteristically the first ATG initiates translation in eucaryots. It is not known where translation initiation occurs in pc309. At the 3' end of the cDNA sequence is an add poly A signal at base 2896. When pc309 is compared to pcPMSS there is a region of 11 bases starting at base 2594 that is absent in pcPMSS. This 11 base difference appears to

be an imperfect duplication of the preceding 8 bases. The 11 base insertion is GGCCGGGGCCT while the preceeding 8 bases are GGCCGCCT. The difference between these sequences is the trimer GGG located after the fifth base in the 11 base sequence. This 11 base insertion interrupts the reading frame. The putative stop translation codon in Sus is positioned two codons before the 11 bp insertion in pc309. At this point strong similarity between pc309 and Sus breaks down, further indicating that this is the region of the cDNA which encodes the stop translation sequence. The positions corresponding to both sides of the 11 base insertion in pc309 may encode the stop translation sequence TAA present in pcPMSS. The same flanking regions may have served as the stop translation codon TGA prior to the 11 base insertion in pc309. The next downstream trimer which could serve as a stop translation codon in pc309 is the sequence TAA at base 2749. This is 156 bases downstream; this extra 156 bases would encode 52 additional amino acids. The resulting sucrose synthase would be about 6% larger, perhaps sufficiently different to be detected on a 7.5% SDS-polyacrylamide gel. Reaction of fractionated protein with anti-Sh1 antibody did not detect a sucrose synthase of around 93,000 mol wt in P. purpureum leaf tissue. Of the five or six P. purpureum sucrose synthase cDNAs isolated only one was p309 encoded. This indicates that p309 is not expressed in leaf tissue to the extent that the structural gene encoding

pcNSS is. This would explain the absence of a sucrose synthase subunit of approximately 93,000 mol wt.

The cDNA sequence of a Sh1 clone was determined to be 2746 bp (Werr et al. 1985). With an open reading frame of 2406 bp, this encodes 802 amino acids. The P. purpureum pc309 cDNA prior to the 11 base insertion would encode 793 amino acids with a 2379 base open reading frame. With the 11 base insertion there is an open reading frame of 2535 bases which would encode 845 amino acids. In both the SDS-polyacrylamide gel (Fig. 1) and the non-denaturing gradient gel (Fig. 2) the Pennisetum proteins appear to be slightly smaller. This may be the case if the P. glaucum sucrose synthase cDNA encodes a 793 amino acid protein and the majority of the sucrose synthase protein in P. purpureum is the same size.

There is little divergence between pc309 and pcPMSS while divergence from the pc309 sequence is extensive in comparison to Sh1 and is intermediate in comparison Sus. The 3' untranslated regions of the two Pennisetum sequences vary little while the maize Sus cDNA exhibits comparatively extensive divergence. A difference of six base pairs in the 3' untranslated region of the Pennisetum cDNA sequences indicates a recent divergence of pc309 and pcPMSS as compared to their divergence from the maize Sus sequence. When pc309 is compared to the pcPMSS sequence in Fig. 35 (including the untranslated 3' region) there is 98.6% similarity; this includes the 11 base insertion present in pc309. Excluding the 11 base

insertion there is 99.1% similarity between these two Pennisetum sequences. When the 106 bases 5' to the second ATG in pc309 at base 217 is compared to the same bases in the Sus sequence there is approximately 86% similarity. When comparing the putative open reading frames of pc309 and Sus there is approximately 92% similarity. In the putative 3' untranslated region of these two cDNA sequences the only detectable similarity is the 75 bases 5' to the poly adenylation signal in pc309; in this region there is approximately 75% similarity between pc309 and Sus cDNA sequences. When pc309 is compared to a Sh1 cDNA sequence the only detectable similarity in the region 5' to the second ATG is an 11 base run centered at base 170 of the pc309 sequence. In the first 50 bases of exon 3 in the pc309 sequence there is approximately 58% similarity to the Sh1 cDNA sequence corresponding to this region. Approximately 70% similarity exist between pc309 and Sh1 cDNA sequences of exon 4. From exon 5 in the Sh1 sequence through exon 15 (Sh1 has 16 exons total) there is approximately 78% similarity. This includes only a segment of the pc309 sequence corresponding to exon 15 of Sus. There is no detectable similarity between the 3' untranslated regions of pc309 and Sh1. The sequence comparison of pc309 to the maize sucrose synthase cDNAs is in agreement with the hybridizational analysis which indicated that p309 was much closer related to Sus than to Sh1. The pc309 and pcPMSS sequences have diverged recently.

CHAPTER 5
DISCUSSION

This study was undertaken to gain molecular evidence concerning the progenitors of the allotetraploid P. purpureum. Previous work has indicated a close relationship between P. purpureum or napiergrass, P. glaucum or pearl millet, and P. squamulatum. Evidence suggesting a close relationship between these species includes cytological evidence for P. purpureum and P. glaucum interspecific hybrids (Harlen 1975; Jauhar 1981), crossability (Dujardin and Hanna 1984; Dujardin and Hanna 1989), isozyme banding patterns (Lagudah and Hanna 1990), restoration of male fertility in pearl millet by interspecific hybridization (Dujardin and Hanna 1990), common seed proteins (Lagudah and Hanna 1990), and relatedness of mtDNAs (Chowdury and Smith). This paper presents additional evidence which strongly suggests that tetraploid P. purpureum, hexaploid P. squamulatum, and diploid P. glaucum share common genomes. Models are presented for the evolutionary pathway leading to P. purpureum.

Varying amounts of Kpn I families of tandemly arrayed repetitive sequences were identified in all Pennisetum species observed. The polyploids P. purpureum and P. squamulatum contain the 140 bp and the 160 bp Kpn I families whereas the

diploid P. glaucum contains only the 140 bp Kpn I family. P. hohenackeri, another diploid, contains only the 160 bp Kpn I family. Other Pennisetum species also contain a 160 bp Kpn I family, although the Kpn I family of P. flaccidum requires detection by hybridization.

Several conclusions can be drawn about the Kpn I families of P. purpureum. The 140 bp Kpn I family is apparently the A' genome marker while the 160 bp Kpn I family is contained within the B genome. The methylated Kpn I families of P. purpureum are most likely the major tandemly arrayed repetitive sequence families within the nucleus. Similarly, the 140 bp Kpn I family of P. glaucum is apparently the major highly repetitive sequence family.

P. purpureum, P. squamulatum, and P. glaucum 140 bp Kpn I families are nearly homologous as shown by DNA sequencing. The chromosomes of pearl millet have been termed homologous or at least homoeologous (partial homology) to the A' genome of napiergrass based upon cytological studies of triploid interspecific hybrids and karyological features (Jauhar 1981). When a triploid interspecific hybrid (AA'B) was treated with colchicine, the resulting synthetic amphidiploid (AAA'A'BB; 2n=6x=42) displayed absent or infrequent multivalent formation during meiosis (Jauhar and Singh 1969). The lack of multivalents is possibly due to preferential pairing between the A-A, A'-A', and B-B genome chromosomes. One possibility is that the A and A' chromosomes of P. glaucum and P. purpureum

are differentiated. Another possibility would be the presence of diploidizing genes which facilitate diploid-like pairing in the allohexaploid (Jauhar 1981). The evidence presented in this paper suggests that the 140 bp Kpn I families of P. purpureum (PI300086), P. squamulatum (PS26), and P. glaucum Tift 23 have diverged recently when compared to the other Pennisetum species examined.

The P. purpureum (PI300086) and P. squamulatum (PS26) 160 bp Kpn I families are more closely related to each other than they are to the P. hohenackeri (PS156) 160 bp Kpn I family. The Pennisetum which contributed the chromosomes of P. purpureum and P. squamulatum containing the 160 bp Kpn I families is unknown. It does not appear to be the diploid P. hohenackeri when the RFLP and isozyme evidence presented here is considered.

The model for the origin of the Kpn I families involves a truncated tRNA progenitor of about 60 bp. This tRNA origin is based upon the presence of RNA polymerase III intergenic promotor elements in these repeats and the similarity between the repeats and a $tRNA^{Arg}$ gene from tobacco chloroplast (Deno and Sukiura 1986). This 60 bp sequence is a subrepeat within the 160 bp Kpn I sequences. After rearrangements which appear to have involved insertions, deletions, and substitutions, and a combination of three subrepeats, a 160 bp Kpn I sequence was formed. This was followed by amplification of this 160 bp sequence which spread through out the genome and perhaps

contributed to the constitutively heterochromatic regions. The 140 bp Kpn I sequence resulted from a deletion of 18 bp within a 160 bp Kpn I sequence followed by preferential amplification of this 140 bp sequence in a diploid genome to a level much greater than that of the 160 bp sequence. The deletion of the 18 bp region interrupts an inverted repeat region which could potentially compose a hairpin of 16 bases.

A Hind III family of repetitive sequences was shown to be present in P. squamulatum but not in P. purpureum. This repeat family does not appear to be tandemly arrayed because of the lack of concatomers. This suggests that P. squamulatum contains additional genetic information not present in P. purpureum. However, the Hind III family in P. squamulatum may exist in P. purpureum in an undigestible form. If P. squamulatum is a progenitor of P. purpureum then one must invoke a loss of chromosomes since P. squamulatum is in the X=9 group of Pennisetum species while P. purpureum is in the X=7 group. This loss of chromosomes could also account for the presence of the Hind III family of repeats present in P. squamulatum but not in P. purpureum.

The presence of 160 bp Kpn I families in P. purpureum and P. hohenackeri suggests a closer relationship than may be the case. When considering comparisons between P. purpureum and P. hohenackeri such as phenotype, isozyme banding patterns, and RFLPs, the conclusion is that the 160 bp Kpn I families present in both these species are a result of parallel

amplification. The B genome progenitor of P. purpureum likely diverged from the P. hohenackeri progenitor earlier in time than the divergence of the P. purpureum A' genome progenitor from P. glaucum or the P. glaucum progenitor. Hence, even though both P. purpureum and P. hohenackeri have common 160 bp Kpn I families they may not be closely related.

Several lines of evidence, previously published and in this paper, indicate a close relationship between P. purpureum and P. squamulatum. Chowdhury et al. (1988) concluded, based upon mtDNA characterization, that P. purpureum, P. squamulatum, and P. glaucum are related but that P. purpureum and P. squamulatum are more closely related. The authors suggested that P. glaucum may be a progenitor of P. purpureum and P. squamulatum. The isozyme banding patterns presented here as well as in previous work suggest a close relationship between P. purpureum and P. squamulatum as well as P. glaucum. Furthermore, the rDNA Sst I RFLPs and the RFLPs of Pennisetum species when probed with cloned P. purpureum genes suggest that P. purpureum and P. squamulatum share common genomes. When considering the above evidence along with the homology of the Kpn I families between P. purpureum and P. squamulatum, a model for the interspecific hybridization event which gave rise to P. purpureum would, out of necessity, include P. squamulatum.

Developing a model for the evolution of P. purpureum involves the consideration of relatedness between P.

purpureum, P. glaucum, and P. squamulatum. P. purpureum and P. squamulatum have many similarities between their genomes and may represent a recent divergence. The mtDNA similarity between these species suggest that P. squamulatum may be the maternal progenitor of P. purpureum. P. purpureum would not be the maternal progenitor of P. squamulatum because napiergrass is in the X=7 group of Pennisetum species while P. squamulatum is in the X=9 group; a loss of chromosomes could occur to where there would be a lower base number. Also the mtDNA data indicate that they both could be the result of recent independent interspecific hybridizations involving the same maternal progenitor. Evidence shown here indicates that the B genome as well as the A genome of P. purpureum may also be present in P. squamulatum.

Evidence published elsewhere and the evidence presented in this paper suggest that P. glaucum and P. squamulatum may share a similar genome in much the same way as P. glaucum and P. purpureum do. Because of this evidence, the hexaploid P. squamulatum should be considered a secondary gene pool species of P. glaucum since it likely contains one genome similar to that of pearl millet.

The conflicting evidence which questions the possibility of P. squamulatum being a progenitor of P. purpureum includes several observations. There are common karyological features between P. purpureum and P. glaucum. P. squamulatum could be a progenitor of P. purpureum only if it is an

autoallohexaploid. Since P. squamulatum is apomictic, an escape from apomixis would be necessary for it to be the maternal progenitor of P. purpureum yet the mtDNA similarity shows that this cytoplasmic genome of P. purpureum is more closely related to P. squamulatum than it is to P. glaucum. P. squamulatum is in the X=9 group of Pennisetum species while P. purpureum is in the X=7 group. If P. squamulatum and P. glaucum are the progenitors of P. purpureum then it is relevant to ask how so many of the molecular characteristics of pearl millet were lost in napiergrass.

There are several karyological features common to both the A genome of P. glaucum and the A' genome of P. purpureum (Jauhar 1981). (1) The shortest chromosomes of the above genomes are associated with the nucleolus. (2) Both species exhibit rapid terminalization of chiasmata during meiotic prophase. (3) Centromeric heterochromatin patterns are similar while some P. purpureum genotypes have terminal knobs on long arms of some chromosomes. These common features of P. purpureum and P. glaucum suggest a recent divergence. It would also indicate that the allotetraploid P. purpureum resulted from an interspecific hybridization between P. glaucum and another diploid through nonreduced gametes because the A chromosomes of pearl millet are so similar to the A' chromosomes of napiergrass.

Interspecific hybridization leading to the appearance of P. squamulatum needs to be considered when addressing the

question of the evolution of P. purpureum. One of the progenitors of P. squamulatum is likely either P. glaucum or a progenitor of P. glaucum. The other genomes of the hexaploid P. squamulatum may have arisen from an autotetraploid or an allotetraploid. Based upon the evidence presented here P. squamulatum may be one of the progenitors of P. purpureum; at the least, they may share common genomes. If P. squamulatum originated as an allohexaploid then it would not be a progenitor of P. purpureum because three different genomes would have been contributed from P. squamulatum to the interspecific hybrid known as napiergrass. However, if the apomictic P. squamulatum evolved as an autoallohexaploid it could be considered a progenitor of P. purpureum. An interspecific hybridization between a P. glaucum type Pennisetum and an autotetraploid through nonreduced gametes would lead to an autoallohexaploid. This autoallohexaploid would likely be apomictic with the apomixis genes coming through the autotetraploid. Autotetraploids are usually apomictic in Pennisetum species (Jauhar 1981). The type of hexaploid which P. squamulatum is considered varies. It has been termed an autoallohexaploid, an allohexaploid, and a segmental allohexaploid. Jauhar (1981) considers P. squamulatum to be a segmental allohexaploid with the formation of multivalents during metaphase and diakinesis of meiosis due to segmental homology of the constituent genomes. If P. squamulatum was originally an autoallohexaploid then

chromosomal differentiation may have led to diploidization; this could have been facilitated if the hypothetical autotetraploid progenitor of P. squamulatum was an intraspecific autotetraploid rather than a tetraploid resulting from a chromosomal doubling. Also, if P. squamulatum contained diploidizing genes, this would cause an autoallohexaploid to behave meiotically as an allohexaploid.

If P. squamulatum is one of the progenitors of P. purpureum then it is likely to be the female progenitor as judged by the similar mtDNA within the two species. But the obligately apomictic P. squamulatum has not been shown to have reduced female gametes, but only reduced male gametes. For P. squamulatum to be the maternal progenitor of napiergrass, an escape from apomixis would be necessary; the hexaploid P. squamulatum reproduces apomictically. The meiotic process in a hexaploid may lead to irregular meiosis. A loss of chromosomes via nondisjunction could account for P. squamulatum being in the X=9 group of Pennisetum species while P. purpureum is in the X=7 group. For P. squamulatum to be the maternal progenitor of P. purpureum the above conditions would have to be met.

The similarity of P. glaucum and P. purpureum chromosomes and the dissimilarities between these two species on the molecular level seem contradictory. On the molecular level P. purpureum is more closely related to P. squamulatum than it is to P. glaucum. This can be explained if the P. purpureum A'

genome evolved from a reduced gamete of the diploid P. glaucum and a reduced gamete of the hexaploid P. squamulatum which evolved from either P. glaucum or a P. glaucum progenitor. The A' genome of P. purpureum would have originated as a heterozygous genome from two different species. Meiotic drive could then have led to an homogenization of the P. purpureum A' chromosomes away from P. glaucum DNA sequences and towards the P. squamulatum DNA sequences. This model would propose that while the karyological features of P. purpureum and P. glaucum are similar, the individual genes have diverged.

While the model for the evolution of P. purpureum through P. squamulatum and P. glaucum seems to invoke unusual circumstances, the molecular evidence presented here suggests that this may be the case. If P. purpureum and P. squamulatum evolved from common progenitors (including P. glaucum or a P. glaucum progenitor) in separate hybridization events then it would be expected that P. purpureum, P. squamulatum, and P. glaucum would all show similar divergence from each other. In contrast, the data suggest that P. purpureum and P. squamulatum diverged more recently than their divergence from P. glaucum. Regardless of the polarity of evolution, P. purpureum is more closely related to P. squamulatum than it is to P. glaucum.

Analysis of Pennisetum sucrose synthase genomic DNA, cDNA, RNA, and protein involved a comparison to maize. The size of the sucrose synthase subunits as well as the non-

denatured sucrose synthase enzyme from the Pennisetum species observed indicated that they may be slightly smaller than their maize counterparts. Sequencing of sucrose synthase cDNAs from P. purpureum and P. glaucum suggests that that is the case.

Probing of P. purpureum DNA with the Sh1 and Sus genomic clones from maize exhibited several cross hybridizing sequences. Four clones were selected from a genomic library which cross hybridized more strongly with Sh1 than with Sus. These clones were composed of two classes. One class cross hybridized to the 3' flanking region of the Sh1 genomic clone whereas the second class cross hybridized to the 5' end of the Sh1 structural gene. One clone (p324) was investigated further. Two genomic clones from P. purpureum which strongly hybridized Sus were isolated. One (p309) was partially sequenced. At least one strand of most of the potential exon/intron boundaries was sequenced and compared to Sus. The clone p309 was similar to Sus in the protein coding region of exon 15 suggesting that p309 did not contain 15 introns as in Sh1. The sequence of the cDNA clone, pc309, had 92% similarity in the protein coding region to a Sus cDNA from maize. No differences between the gene structure of these two sucrose synthase genomic sequences were found.

When P. purpureum and P. glaucum RNAs were probed with sucrose synthase coding sequences from maize and P. purpureum unexpected results were obtained. Probing of P. purpureum leaf

RNA and P. glaucum seed RNA with Sh1 and Sus genomic clones showed that the majority of the sequences which hybridized the maize sucrose synthase genes were in the 1.6 kb to 2.1 kb size range. As expected, there were also Pennisetum sequences in the 2.8 kb size range which hybridized the maize probes. When P. purpureum leaf RNA was probed with the genomic clone p309, RNA in the 1.9 kb size range hybridized by far the most probe, followed by RNA in the 1.6 kb size range. The majority of the likely sucrose synthase mRNAs seen with the p309 probe migrated at an intermediate position relative to the Sh1 and Sus transcripts. The question of the protein encoded by the 2.1 kb and smaller RNAs remains to be determined.

Since the poly A^+ mRNA hybridizes to the sucrose synthase genomic clones mentioned above, there exists the possibility of non-sucrose synthase proteins binding to anti-Sh1 antibodies. The SDS-polyacrylamide fractionated P. purpureum leaf proteins were degraded extensively during the protein isolation; this caused the antibody to bind to what appeared to be sucrose synthase degradation products. When P. purpureum root proteins were fractionated on a gradient gel and reacted with anti-Sh1 antibodies, only high molecular weight protein was observed. However, the high levels of 1.9 kb RNA hybridizing p309 were observed in leaf tissue of P. purpureum; intact protein from leaf tissue was not reacted with anti-Sh1 antibody. So it is not known if the protein encoded by the 1.9 kb mRNA cross reacts with anti-Sh1 antibody. The 1.9 kb RNA is

not encoded by p309; p309 encodes sucrose synthase as shown by sequencing. The 1.9 kb poly A^+ RNA may be encoded by the P. purpureum genomic clone p324.

The P. purpureum genomic clone p324 has similarity to exons 1 and 2 of the genomic clone Sh1 as well as to a restriction fragment containing exons 3 through 7. A restriction fragment from the genomic clone Sus containing exons 4 through 7 also hybridizes to p324. This hybridization pattern of p324 indicates that it has similarity to at least the first four exons of sucrose synthase. The hybridization patterns of P. purpureum poly A^+ RNA probed with p324 and p309 are the same, both clones hybridize most strongly to the 1.9 kb RNA. A size-fractionated cDNA library was screened with a p309 restriction fragment containing exons 5 through 13; only putative sucrose synthase cDNAs hybridized to the probe. When those same lifts were screened with the nearly full length sucrose synthase cDNA clone pcNSS from P. purpureum, about 1.4% of the lambda clones containing greater than 1.8 kb inserts hybridized to the probe. This suggests that there are high copy number transcripts which have similarity to the 5' end of sucrose synthase. These abundant cDNAs have been isolated. The possibility exists that the genomic clone p324 encodes this abundant transcript.

Two sucrose synthase cDNAs were isolated from the tetraploid P. purpureum; these are distinct from each other. The most abundant cDNA was termed pcNSS while the least

abundant was the pc309 clone. Since P. purpureum is an allotetraploid, the presence of these two distinct cDNA clones does not indicate that there is a dual sucrose synthase system. There is no indication whether these clones are from the same or different genomes. Both pc309 and pcNSS were used to probe Pennisetum DNAs. When comparing the hybridization patterns of the two probes on identical blots, it is apparent that these probes hybridize to different sequences within each of the Pennisetum species observed. When the diploid P. glaucum hybridization patterns are compared to the tetraploid P. purpureum hybridization patterns there are roughly twice as many strongly hybridizing sequences in the tetraploid as compared to the diploid. These data suggest that there may be a dual sucrose synthase system in the Pennisetum species examined. The tissue specificity may be different. The most abundant cDNA in the P. purpureum leaf, pcNSS, was found to have closer similarity to Sh1 from maize (up regulated in the endosperm) than the least abundant cDNA, pc309. The selected cDNA from the P. glaucum seed library had stronger similarity to Sus from maize which is the least abundant sucrose synthase protein in maize seed; however the pcPMSS cDNA was selected due to strong hybridizational intensity with the p309 probe so it could possibly encode the least expressed protein in pearl millet seed. These data also suggest that both genomes of P. purpureum may contain the two types of sucrose synthase genes encoding pc309 and pcNSS type cDNAs. There were two Sus

hybridizing genomic sequences cloned from P. purpureum, p308 and p309. However, there were no genomic sequences cloned from P. purpureum which correspond to the cDNA pcNSS. The sucrose synthase coding sequences from P. purpureum have similarity to the maize genes, especially p309. However, there are distinct differences, for example pcNSS appears to be a hybrid between the two maize sucrose synthase genes. Also, there could be differences in the expression of the sucrose synthase genes in P. purpureum as compared to maize.

The sequencing of the P. purpureum cDNA clone pc309 and the P. glaucum cDNA clone pcPMSS shows the strong similarity between these two sequences. Since P. glaucum is considered a progenitor of P. purpureum, the possibility of p309 being located in the A genome of napiergrass and originating from pearl millet can be considered. Since the B genome of P. purpureum has diverged considerably from the A' genome of P. purpureum and the A genome of P. glaucum it is possible that p309 is within the A genome of P. purpureum. This would support the close similarity between pc309 and pcPMSS. This is the first sequence data on sucrose synthase cDNA clones from two species within the same genus. The close similarity between these two clones from P. purpureum and P. glaucum may be due to these species being in the same genus or, as mentioned above, it may be due to P. glaucum being a potential progenitor of P. purpureum.

Comparison of the intron sequences of p309 and Sus led to surprising results. There is a gradient effect of sequence similarity which not only includes the introns but also the 5' and 3' untranslated portions of the cDNAs from both of these sucrose synthase clones. The putative 5' untranslated region of pc309 and Sus cDNA exhibit greater similarity than the 3' untranslated regions. The introns towards the 5' end of the structural gene exhibit greater similarity than the introns at the 3' end of the gene, from 79% in intron 3 to no detectable similarity in exons 13 and 14. The protein coding regions of p309 and Sus do not exhibit this type of variation in similarity. This sequence divergence appears to involve more than natural selection and genetic drift; however, no explanation is presented here. This gradient effect involving intron similarity will be investigated further.

The maize cDNA clone Sus has 92% similarity to pc309 in the putative protein coding region of the cDNA; the similarity diminishes after the potential stop translation codon in Sus. This is not the case when pc309 and pcPMSS are compared. The similarity continues in the 3' untranslated region of the cDNAs. The overlapping regions of the cDNA clones pc309 and pcPMSS exhibit 98.6% similarity. This strong similarity between pc309 and pcPMSS indicates a recent divergence. At the same time the similarity between pc309 and the maize Sus cDNA shows that these coding sequences stem from a common progenitor sequence as well. There is a distinct maize Sus

counterpart in P. purpureum and P. glaucum; but there does not appear to be a distinct Sh1 counterpart in P. purpureum.

The sequence data from the Kpn I families of tandemly arrayed repetitive elements and the sucrose synthase cDNAs from P. purpureum and P. glaucum support the proposed role of pearl millet as a progenitor of napiergrass. At the same time, the relationship of P. purpureum to P. squamulatum is quite strong. Examination of P. squamulatum and P. glaucum sucrose synthase genomic sequences corresponding to p309 could easily be facilitated by the use of polymerase chain reaction. This would indicate if p309 came through P. squamulatum or P. glaucum.

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BIOGRAPHICAL SKETCH

Where I am from and what I have done is not so important as who I am today and where I am going. This is what I prefer to concentrate on.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

L. Curtis Hannah, Chair
Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Wayne W. Hanna
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Robert J. Ferl
Professor of Horticultural Science

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Don R. McCarty
Assistant Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1990

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